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Proceedings of the 22nd Annual Aquatic Toxicity Workshop: October 2-4, 1995, St. Andrews, New Brunswick

K. Haya and A. J. Niimi (Editors)

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PREFACE/PREFACE

The 22nd Annual Aquatic Toxicity Workshop was held at the Algonquin Hotel in St. Andrews, New Brunswick, October 2 to 4, 1995. The Workshop included 4 plenary presentations, 87 platform presentations and 28 papers in poster sessions. Total attendance was 222. An Environmental Effects Monitoring Symposium was held on October 5 and was organized by Environment Canada.

The 22nd Annual Aquatic Toxicity Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology. It covered topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the Proceedings are published with the support of the Department of Fisheries and Oceans.

Le 22eme atelier annuel sur la toxicité a eu lieu L'Hôtel Algonquin, St. Andrews, Nouveau Brunswick les 2 au 4 octobre 1995. Le atelier a donné lieu a 4 communications lors de séances plénières, 87 exposés d'invités d'honneur 28 communications par affichage. 222 personnes ont assisté au atelier. Un Conférence sur le Surveillance des Effets Environnementals, organisé par Environnement Canada, été mise en pied le 5 octobre 1995.

Le 22eme atelier annuel sur la toxicité aquatique a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministère des Pêches et Océans.

EDITORS' COMMENTS/REMARQUES DES EDITEURS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstracts received limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the authors and are neither condoned or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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An informal look at the parents of Canadian aquatic toxicology

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AN INFORMAL LOOK AT THE PARENTS OF CANADIAN AQUATIC TOXICOLOGY.

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Abstract

Dr. Donald F. Alderdice is the father of Canadian aquatic toxicology, starting that work in 1953, and continuing through a career at the Biological Station in Nanaimo, B.C. Upon retirement he involved himself in an environmental struggle about water diversion. Other "parents" are nominated as those who worked in the discipline for some time, starting before 1965. John H. Neil was apparently the first person employed as a full-time water pollution biologist (1952), for the Ontario government. He did toxicology, rose into administration, then started companies for production and use of aquatic resources. John B. Sprague did water pollution and toxicology studies as a graduate student starting in 1953, then at the Biological Station, St. Andrews, N.B., Univ. of Guelph, and as a consultant. Gerard Leduc started his toxicology with a master's degree in 1958, and continued with the Québec government, Concordia Univ., and retired early to engage in archeology. Terry E. Howard came from Britain in the early 1960s to work on toxicity of pulp mill and other wastes at B.C. Research, eventually moving into management of that and other companies. James A. Servizi did 30 years of toxicology starting in 1963, for the International Pacific Salmon Commission and government; upon retirement he enrolled in a college dramatics program.

Other people worked in closely associated fields, or for short periods. Thomas W. Beak deserves a special place as the first full-time consulting pollution biologist (mid-1950s), whose company grew and did much toxicology. A dozen other people published research on aquatic toxicity in the 1950s and 1960s but moved on to other fields. Another dozen or so were involved in studying forest-spraying with insecticide as it impinged on their fisheries work. A famous group of Canadians worked with F.E.J. Fry on relations of fish to temperature and oxygen, but probably considered themselves fish physiologists rather than toxicologists.

It is a remarkable contrast to see the hundreds of Canadians involved in aquatic toxicology today, compared to surprisingly few in the old days. It is difficult for an old-timer to believe that the 22nd Annual Aquatic Toxicity Workshop has grown to three simultaneous sessions, with 200 advanced registrants.

This paper may help in re-introducing ourselves to the Canadian aquatic toxicologists who existed in the 1950s and before 1965, thirty-plus years ago. I am designating them as PARENTS OF CANADIAN AQUATIC TOXICOLOGY, although it turns out they were all fathers. For the young people at this workshop, perhaps it will be good for your soul to reflect upon the roots of your profession. It is also interesting to note a strong connection of this St. Andrews Biological Station with early Canadian work on water pollution and aquatic toxicology, a connection that continues. The information is presented in a somewhat light-hearted fashion, but I hope I have not misrepresented anyone.

Six people were definitely working in aquatic toxicity before 1965, and stayed in it for some time, qualifying for the "Parents" list in my opinion. After them, I will cover some people who entered the toxicity field but did not stay, then some people in associated fields of water pollution and fisheries. I have listed some publications up to 1965, but have not attempted to cover life-lists.

The parents

Donald F. Alderdice

This would seem to be the genuine father of Canadian aquatic toxicology, judging by his early activity and his engagement over decades. In 1951, Don Alderdice was in the Maritimes, doing field work on fisheries and DDT spraying of forests. In 1953 he went to the Biological Station at Nanaimo, to work for the Fisheries Research Board of Canada (F.R.B.). That year, he started toxicity tests on insecticides, as alternatives to DDT. He built an aquatic toxicity lab, broadened to work on pulp mill wastes and other things, and published four papers on this as senior author with colleagues, in the late 1950s (see References). Fig. 1 shows Don in the 1950s.

Fig. 1. The father of Canadian aquatic toxicology. Dr. D.F. Alderdice in the 1950s.

Don did his PhD at Toronto, with some sophisticated work on three-dimensional response surfaces, showing effects of temperature, salinity and oxygen on the toxicity of pentachlorophenol (Alderdice 1963). He continued in this vein, exercising his talents in mathematics with publications on multi-variate analysis of environmental entities acting on fishes. In particular, he focused on effects on eggs and developing young, work which he had started in the 1950s (Alderdice *et al.* 1958, Alderdice and Wickett 1958). Before he retired from the Dept. of Fisheries in about 1988, he worked on effects of supersaturation of gases caused by power-dams.

What happened to him? He became an environmental crusader! In particular he worked against a plan for diversion of water from a salmon river, for power in north-western British Columbia. He worked with a colleague from Nanaimo, Dr. Gordon Hartman, and Fig. 2 shows them in 1993 when they were in the middle of that struggle. The picture is taken from an article in Harrowsmith (December 1993). They won, when the provincial government revoked the diversion scheme. Don and wife Jean live outside Nanaimo, B.C., and he enjoys the power of his personal computer.

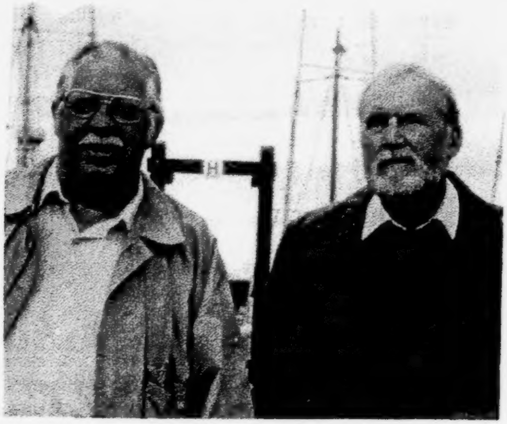

Fig. 2 Dr. D.F. Alderdice (left) and Dr. Gordon Hartman in 1993, retired and campaigning environmentally in B.C.

John H. Neil

John seems to be the first person in Canada who was employed as a full-time pollution biologist, starting in 1952 with the Ontario Dept. of Health. In that capacity he did the biological field work for the first really big Canadian study of water pollution, in the Spanish River (Dymond and Delaporte 1952). John soon branched into fish toxicity and tested many industrial wastes in his Toronto laboratory in the 1950s and early 1960s. His master's degree at Univ. of Toronto was an early piece of work on *sublethal* fish toxicology (Neil 1957). Fig. 3 shows him at a more mature stage.

One of John's problems was success and he found himself promoted out of the toxicity lab, as his agency changed itself to the Ontario Water Resources Commission, and finally to part of the Ministry of the Environment. He was successively supervisor of the Biology Branch, Director of the Division of Laboratories, then Director of the Water Resources Branch of M.O.E.

He left in 1974 to start his own company, *Limnos Ltd.* John says "I suppose my first love has always been utilization of aquatic resources ... and the wish to return to my roots led me to leave the government". His company conducted research on fish and fisheries, aquatic plants and algae, waste treatment, general limnology and the use of aquatic resources for beneficial purposes. One of the latter was development of livestock feed from aquatic weeds and other waste products. In the early 1980s he spent five years putting together a source of warmed water from Ontario Hydro with a de-commissioned sewage plant and outside financing, to form *Coolwater Farms Ltd.* which is the largest producer of cultured trout in Canada and does associated research.

Fig. 3 J.H. Neil at the time of operating aquatic resources companies.

What happened to him? John is still active in the company. He and his wife Jeanne have cruised Georgian Bay for 37 years in an east coast lobster boat.

John B. Sprague

I will nominate myself as another father of Canadian aquatic toxicology. Like most others in the early days, I was a water pollution biologist and did field surveys as well as lab toxicity. I started in 1953 with a study of the enriched/polluted Avon River in Ontario, for a master's degree at Toronto. That had been inspired by field surveys the previous summer, which suggested water pollution as an interesting and useful field (and also rather weird, at that time). PhD research at Toronto with Dr. Fred P. Ide was lab work on tolerance of low oxygen and high temperature by crustaceans. My supervisory committee forced me to take courses in public health and pharmacology, and some of it turned out to be very beneficial! For example I became one of the few aquatic people who had taken a formal graduate course in pharmacological bioassays and their analysis.

In 1958 there was a miracle, and Dr. John L. Hart, director at the St. Andrews Biological Station of F.R.B., offered a chance to start a section on water pollution research. (Earlier, when director of the Nanaimo station, Dr. Hart hired Dr. Michael Waldichuk, an oceanographer, to start a pollution group. Dr. Hart thus started the first two water pollution research groups that I know of in the federal government.) I stayed at St. Andrews until 1970, doing field surveys on pulp mills and mines in N.B., mine waste in Labrador, and other things. The picture in Fig. 4 was taken during a survey of benthic organisms in 1960; at that time it was socially acceptable to smoke a pipe. Lab work on toxicity started in a rush in 1960, when base-metal mining affected salmon in the Miramichi River. Several projects co-operated with salmon scientists Paul F. Elson and Richard L. Saunders. My publications were slow to emerge but eventually did so (see References, seven from 1963 to 1965).

In 1970 I moved to the Dept. of Zoology at Univ. of Guelph and stayed until 1988. I taught everything from graduate ecology to first-year zoology for 700 students, but best enjoyed a senior course in water pollution biology. Most of my graduate students did lab toxicology, especially the effects of modifying factors. Many went on to great things, and it was always intriguing when a year or so after they graduated, I sat on committees which they chaired. After early retirement in 1988, consulting jobs are providing years of fun, nice trips, and finally, some serious income!

What happened to him? In 1993 Lois and I sold out in Guelph and spent a year sailing down the east coast and back, wintering in the Gulf of Mexico and the Keys. Then we trundled across the country to B.C., to live on an idyllic Gulf Island.

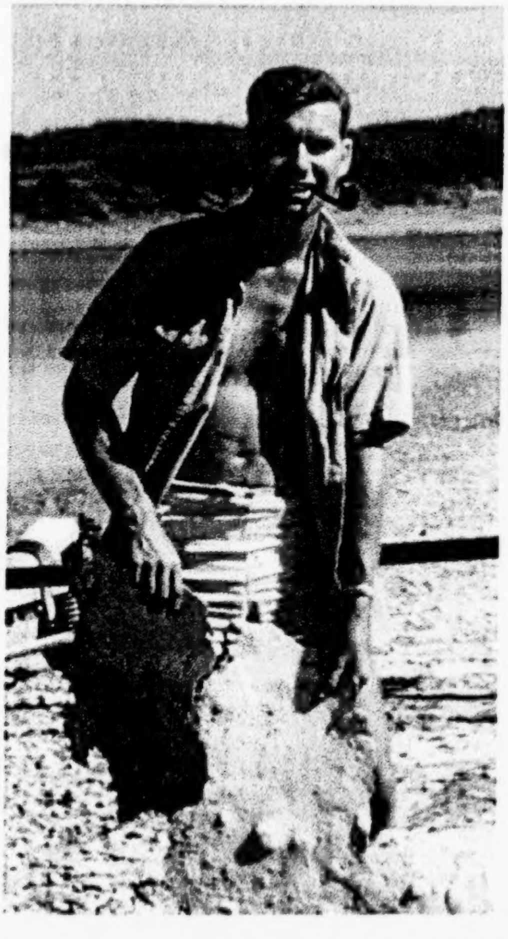

Fig. 4. Dr. J.B. Sprague holding up dried fibre on the upper St. John River, N.B., in 1960.

Gerard Leduc

Gerard obtained his master's degree in 1958, with a thesis on the effects of Toxaphene and freshwater clams. A little later he included his work on Toxaphene and trout in a paper published by Prévost (1960). At that time, Gerard was working for the Québec Biological Bureau. In the early 1960s he obtained his PhD with Dr. Peter

Doudoroff at Oregon State University (Doudoroff *et al.* 1966). He was with the Québec Wildlife Service from 1963 to 1966, still dealing with toxicity and pollution problems.

He joined the faculty of Biological Sciences at Sir George Williams Univ., now Concordia University. He became chairman of the department and built a large aquatic toxicity lab for it. The picture in Fig. 5 shows him in his role as a university professor. Many of his graduate students are well known today in the field of aquatic toxicology. Gerard's special field was toxicity of cyanides and metallo-cyanides, for which he has many publications. He became a North American authority on cyanides and has been an expert witness in various court cases involving cyanide and gold mining.

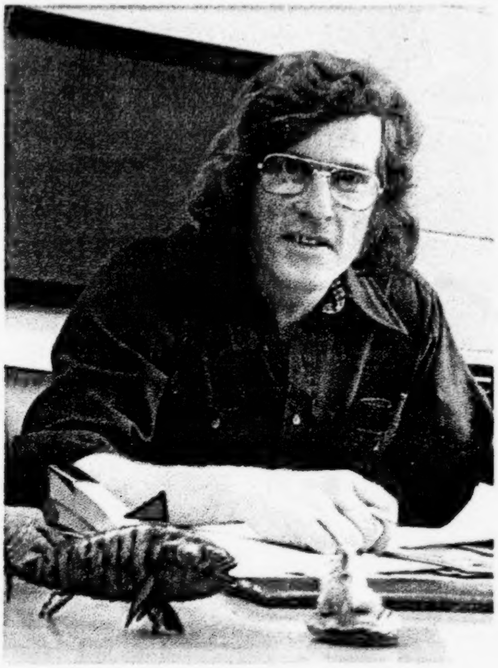

Fig. 5. Dr. Gerard Leduc in his university office about 1978.

What happened to him? Dr. Leduc became absorbed in archaeology, especially in connection with North American native peoples. He retired early to live in les Cantons de l'est of Québec, where he is a spark plug for local historical and touristic projects, and Indian archaeology on a wider scale. Gerard was at an archeology convention in B.C. in May of 1995, visited us on Salt Spring Island, and demonstrated a taste for climbing the toughest local mountain and looking for Indian petroglyphs.

Terry E. Howard

Terry says he was attracted to Canada from Britain, by the pioneering work of Don Alderdice and Rollie Brett in Nanaimo. His first work on toxicity was a contract in the early 1960s, comparing the effects of ionic, colloidal and bound copper on the snails that host schistosomes. In the early and middle 1960s he was in a team at B.C. Research, that estimated threshold concentrations of pulp mill effluent for short-term lethal and sublethal effects. Some of the sublethal work involved buccal pressures in fish to indicate effects on respiratory movements. He stayed with toxicity and his group also spread into waste treatment and in-plant pollution control. The picture in Fig. 6 is from that era, and was taken from a photocopy of a pulp mill paper. Reports were produced during that period, but the first published paper was Howard and Walden (1965).

Terry left the toxicology field in 1980 to involve himself in management of small companies competing in world markets, and was president of *Techwest Industries*. In 1984 he went back to B.C. Research as president, and stayed until 1993.

What happened to him? Terry Howard is still at it. He is partner of *Thin Kerf Technologies Inc.*, which designs and markets instruments to align and upgrade the performance of sawmills in North America and Asia. Terry says: "My career in research was made enjoyable by those scientists and other smart people from universities, government or industry who were willing to help, to share information and to act as colleagues". He and his wife Gael live south of Vancouver.

Fig. 6. Terry E. Howard, reproduced from a paper published in 1981.

James A. Servizi

Jim is known to all for the toxicity lab he ran at the International Pacific Salmon Commission, in Cultus Lake, B.C. He went there in 1963 and retired in 1993, by that time having been absorbed into the Dept. of Fisheries and Oceans (photo in Fig. 7). He started right in on pulp mill toxicity in 1963, working with effluent that simulated the waste from one of the controversial bleached kraft mills on the Fraser River. He and his colleagues assessed effects on adult, juvenile, and egg-stages of sockeye, and means of detoxification. If you ever worked with the Canadian pulp and paper effluent regulations from 1971, you may have wondered where those magic numbers came from that 80 % of a sample of rainbow trout must survive for four days in 65 % concentration of effluent. Someone derived those numbers from the work of Servizi and colleagues, since that appeared to represent state-of-the-art waste treatment in those days. That research of Jim's was eventually published (Servizi *et al.* 1966).

After that a string of similar research projects came along through the decades, many of them centred on chemical and toxicological identification of the active components of pulp mill waste, the effectiveness of various methods of treatment, and physiological symptoms in salmonids. Jim and his co-workers also assessed the toxicity of chlorinated organics in pulp mills about two decades before such studies became a trend in toxicology.

Fig. 7. Dr. James A. Servizi.

What happened to him? Jim retired in 1993, and made quite a change. He is now in his third year at Fraser Valley College, in the Dramatics Dept.! He and his wife are active in theatrical productions, and also bike, hike, fly fish, and volunteer teach in local schools.

A notable consulting biologist

Thomas W. Beak deserves a special place in this little history. He was not a toxicologist, but was Canada's first full-time consultant in water pollution biology, and opened many areas for the rest of us. Tom came from the U.K. to Canada in the middle 1950s. He was actually educated as a chemist but had been drawn into biology from work for British river boards.

Tom talked industries into biological surveys, at first chemical plants and pulp mills in Ontario. He developed an early biotic index, and showed industries how to plot survey results on control charts to see if waste discharge was within acceptable ecological limits (Beak, 1965). The picture of Tom (Fig. 8) is from those early days. It is a winter sampling expedition near the C.I.L. plant at Millhaven on Lake Ontario, and Tom had just dragged his sampling gear on a sled, out through a snowstorm, looking rather like Scott of the Antarctic.

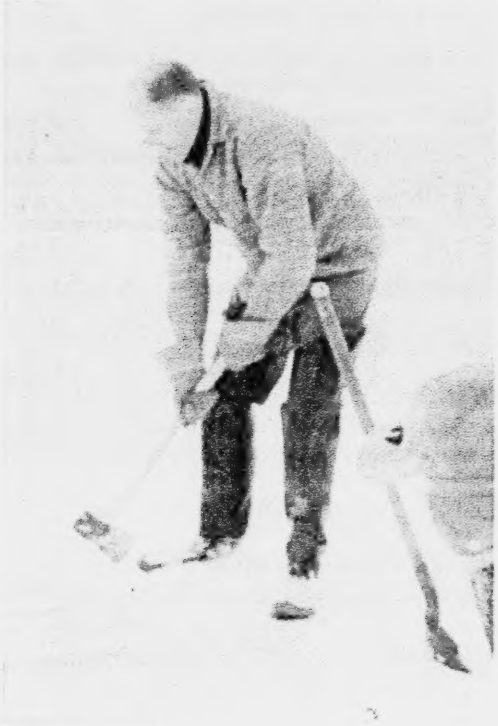

Fig. 8. Thomas W. Beak chopping a hole in the ice of Lake Ontario for sampling benthic invertebrates, on February 21, 1958.

In those early days, Tom did some tests with caged fish, but no lab work on toxicity. He did write a paper involving a good deal of toxicity information (Beak 1958). At first he worked out of a basement office and lab, with his wife Joan taking some of the wide range of work that comes with a two- or three-person operation. (At that time we were trying to arrange employment for me, but the Beak operation could not match $5800 that the government had offered.) Of course, Beak grew into a company, he sold it, it continued to grow, and retains its roots in water pollution and toxicology.

What happened to him? When the company was sold, Tom and Joan Beak sailed off in their yacht to run their coconut plantation in the Seychelles Islands. Unfortunately there were problems such as revolutions, so they sailed back and took up residence in the U.S.A. After a short career in tourism, they devoted themselves to a mission in Africa, helping people develop wells and other necessities for villages. Their address changed a few years ago and I do not know the present status.

Some early but short-term aquatic toxicologists

Several people worked in aquatic toxicity but did not stay, and so are not included here as continuing professionals in the field. Some of them did toxicity work when the topic impinged on their main field of interest.

A.P. Knight could be considered a part-time grandfather of Canadian toxicology. He was a professor at Queens Univ., but was also hired in summers of 1900 to 1907 by the Director of the Marine Biological Station at St. Andrews, N.B., and by the Dominion Commissioner of Fisheries. He did many things in fisheries including some toxicity tests. In New Brunswick, he found that sawdust waste was not lethal to fish, pulp mill effluent was lethal at 10% [a value that generally held true until the 1980s], gas works effluent at 0.5%, and a nail factory effluent [!] at 0.1%. Prof. Knight was a good toxicologist, and used a control fish [yes, a fish]. He also anticipated the documents outlining methods for testing, and he tried various volumes of testwater, finding that with 1 mL of water per gram of fish, they lived for 5 minutes. It appears that we have improved our methods since then.

Other early people with a connection to the St. Andrews station were **D.G. (Dick) Wilder**, a long-time scientist at the station who published a paper (1952) on toxicity of metals to lobsters, in relation to shipping procedures. **Frances Premdas** and **John Anderson** published (1963) on uptake and detoxification of DDT by salmon; Anderson was director of the station for a period in the 1960s.

At the Québec Biological Bureau, **Gustave Prévost** was a driving force in many fields including toxicity of pesticides to fish (Prévost et al 1948). He had retired to St. Jovite by 1981. **Florian Grenier** was an investigator in the agency at that time, and published an early paper on a periodic siphon for fish toxicity tests (Grenier 1960), a distinct improvement over the static test in a glass carboy or pickle jar. Florian moved out of this field, and went to the Québec city aquarium. **André Gagnon** of the Québec Dept. of Fisheries published an excellent paper (1958) on toxicity of DDT to salmon and trout.

Others Canadians who published on aquatic toxicity before 1965 were **G.E. Stringer and R.G. McMynn** (1958) concerning Toxaphene as a fish poison, **S. McDonald** (1962) on detection of insecticides with *Gammarus*, and **R.M. (Bob) Christie and Helen I. Battle** (1963) on histological effects of the lampricide TFM.

Pollutional and related work

Several fields of work and schools of biology are not aquatic toxicology, strictly speaking, but are closely related. People involved in such early work deserve some mention here. The following summary does not attempt to cover any internal reports that might exist.

Some early work involved field studies. A classic tale of gross pollution is quite a shocker to read, with tonnes of fibre discharged and lying deep on the bottom, areas of zero oxygen in the river, etc. (Dymond and Delaporte 1952). Percy Wickett (1959) studied siltation and its effects on salmon spawning in west-coast streams. Gorham and Gordon (1963 assessed the effects of smelters at Sudbury on aquatic vegetation. Forest spraying with DDT involved Canadian aquatic scientists in several parts of Canada. In New Brunswick, many papers came out of the St. Andrews station from 1955 onwards, from the work of Paul F. Elson, C. Jim Kerswill, Miles H.A. Keenleyside, and Fred P. Ide. The work from St. Andrews played a part in the environmental revolution of the 1960s; in the book "Silent Spring" by Rachel Carson, which started a popular environmental movement, chapter 6 was the story of DDT and salmon rivers of northern New Brunswick. The reference list shows two publications by Ide, two with senior author Kerswill, and three by Keenleyside. Papers came from other parts of Canada on effects of DDT spraying on fishes and aquatic insects: Ray R. Langford (1949) from Ontario, Gabriel Filteau (1959) from Québec, Crouter and Vernon (1959) and Todd and Jackson (1961) from British Columbia.

It was not clear what category to use for the work of Morden W. Smith (1935, 1939), unless it is reverse toxicology. In those days, one could write about copper sulphate and rotenone as a means of "eradicating the ... fish population of a lake".

A related field of endeavour that cannot be adequately covered here is the Canadian school of temperature and oxygen relationships for fish, that developed from 1946 onwards with Dr. F.E.J. Fry and his colleagues and numerous graduate students at the Univ. of Toronto. Much of this work is closely related to aquatic toxicology, but is not really part of it. Nor would the investigators have considered themselves primarily as aquatic toxicologists, but more probably as fish physiologists. The people involved in this work read like a who's who of Canadian aquatic biologists, and I collected five typewritten pages of references, just for the early days. They are not given here, but most of them are listed in a manuscript report (Sprague 1964). I have included one reference to Shepard (1955) who studied tolerance of low oxygen using methods like those in aquatic toxicology. Others who studied fish performance in relation to oxygen, clearly relevant to pollution, included J. Rollie Brett, Richard L. Saunders and F.W.H. Beamish.

Conclusion

Is there a moral to this tale? I'm not sure. In this time of cutbacks, we may feel understaffed to deal with the multitude of toxicity problems in the country's waters. It is not clear how that relates to the 1950s when there were perhaps half a dozen people in the country who considered themselves water pollution biologists and/or toxicologists. And that was a time when mills discharged raw seffluent with abandon, and society soaked forest ecosystems with DDT. Perhaps society is lucky to have scraped through that period, with its pickle-jar toxicologists.

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Many current studies of biological effects of industrial effluents are possible only because of the tremendous progress in reducing contaminant loads over the past few decades. Science now routinely searches for chronic effects on relatively fragile organisms in effluents that a few years ago were quickly lethal to more robust species of test animals. Similar changes are occurring in studies conducted in the ambient environment. Identifying and tracking increasingly subtle effects involves application of increasingly sophisticated knowledge. For that knowledge to be used effectively in industrial decision making, an ability to communicate science clearly and precisely among all interested parties is essential.

Improvements in the water and sediment quality in the St. Clair River clearly relate to decreases in discharges over five decades. Further improvements will rely on appropriate risk assessment and priority setting based on the state-of-the-art studies now underway. A review of the progress to data demonstrates that building an increasingly sophisticated understanding of effects monitoring among environmental decision makers has been a key driving force for change. Continuing to build on that knowledge base will be essential to ensure appropriate responses to remaining challenges.

ENVIRONMENTAL RESEARCH? WHAT'S REQUIRED? WHO'S GOING TO DRIVE IT?

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Environmental research needs for refinery and petrochemical plants are examined. National industry associations and plant scientists and engineers were queried about what environmental information is required by their industry. Questions answered and examined include: What environmental concerns which may be answered by research require answers by your plant or industry? Are you performing this research within 5 years? 10 years? Who will develop this information for you...academia?...private sector? How will this information be used?

In today's competitive economy, ways of understanding and improving environmental quality require new and, perhaps innovative, approaches to conducting research. What kind of research is meaningful from an industry perspective? How and to whom does one communicate the information? How are partnerships forged?

TEMPORAL AND SPATIAL TRENDS IN ORGANOCHLORINE CHEMICALS IN THE ST. CLAIR RIVER.

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The freshwater mussel, *Elliptio complanata*, was used to monitor chlorinated chemicals from industrial sources in St. Clair River water. Pentachlorobenzene, hexachlorobenzene and octachlorostyrene were quantified in mussel tissue using electron capture gas chromatography. Temporal trends in organochlorine concentrations in mussel tissues and St. Clair River water were similar. Water data also indicate that pulse loadings have decreased in frequency over time. Mussels deployed in wetlands adjacent to the St. Clair River accumulated less hexachlorobenzene and octachlorostyrene than mussels deployed in the St. Clair River. Wetland deployed mussels accumulated similar amounts of DDE and PCB as St. Clair River mussels. This suggests that wetlands are buffered with respect to chemical contamination from upstream point sources but are more susceptible to chemical contamination due to long range deposition or historic direct application. The environmental fate of St. Clair River chemicals was determined for fish and invertebrate species from Lake St. Clair and Lake Erie.

INTEGRATING CAEAL AND THE REGULATORY SYSTEM FOR BIOASSAY TESTING IN CANADA

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The Canadian Association for Environmental Analytical Laboratories (CAEAL) is a nonprofit organization which offers accreditation to environmental laboratories. A system of certification and accreditation was recently developed for bioassay testing laboratories across Canada. Laboratories may now be accredited for the standardized acute lethality tests with rainbow trout and *Daphnia magna*, which are used by the provincial and federal governments for regulatory purposes. In Ontario, accreditation means that laboratories comply with Canadian standards for laboratory practice, that culturing and holding facilities meet criteria set by the Ontario Ministry of Agriculture and Food, and that the test results will stand up in a court of law.

The extent to which CAEAL has been embraced by government, testing laboratories and industry differs greatly. As a result of these differences, neither industry nor government receives the full benefits of an accreditation system. In Ontario, there is no requirement for laboratories performing provincially mandated testing to be accredited, and the reporting requirements relating to this testing is minimal. In contrast, testing under federal regulations (e.g. the Pulp and Paper Effluent Regulations) requires extensive reporting and full disclosure of all QA/QC data. While there is no absolute requirement for laboratories performing these tests to be accredited, some federal publications mention CAEAL's role in setting standards and quality criteria for aquatic toxicity laboratories. Deficiencies in these two models will be identified and a new model for maximizing the benefits of accreditation will be proposed.

TOXICOLOGY AND CHEMISTRY IN WATERSHED MANAGEMENT
SESSION CHAIR: P.-Y. CAUX

AQUATIC TOXICITY AND ENVIRONMENTAL IMPACT OF SANITARY LANDFILL LEACHATE DISCHARGES IN THE SACKVILLE RIVER, NS

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The final leachate from the Highway 101 Landfill, Sackville, NS, and water in the Sackville River was chemically characterized and evaluated for acute and sub-lethal aquatic toxicity. In addition, the impact of leachate discharges on the benthic macroinvertebrate community was determined. The landfill uses a wetland tertiary treatment system and receives solid waste from the municipalities of Halifax, Dartmouth, Bedford and the County of Halifax, NS. Samples were not acutely toxic to rainbow trout (*Oncorhynchus mykiss*), *Daphnia magna* and *Vibrio fischeri*. There was no detrimental effect on the survival and reproduction of *Ceriodaphnia dubia*, although a slight effect on reproduction was detected in river water at the control station (700 m upstream). Moderate toxicity to *Selenastrum capricornutum* was detected at the control and at station 2 (40 m from the outfall). Ames tests revealed no mutagenic or cytotoxic effects in the final leachate or in river water downstream; the control sample was slightly cytotoxic however. Benthic macroinvertebrate investigations revealed that the leachate discharge had a localized impact on community structure near the outfall, but by 50 m downstream, the conditions were approaching those upstream of the discharge point. The tertiary treatment system used at the landfill produced a non-toxic leachate that doesn't have widespread or long-term impacts on aquatic life in the Sackville River.

INFLUENCE OF SPECIES AND SEX ON CONCENTRATIONS OF ORGANIC CONTAMINANTS IN FRESHWATER MUSSELS FROM THE ST. LAWRENCE RIVER, WITH IMPLICATIONS FOR BIOMONITORING PROGRAMS

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Biological factors may significantly influence the accumulation of environmental contaminants by mussels, and must therefore be controlled to achieve good sensitivity and precision in "mussel watch" programs. This study examined the influence of species and sex on concentrations of organochlorine pesticides, chlorobenzenes, PCBs and PAHs in *Elliptio complanata* and *Lampsilis radiata radiata* from 12 sites on the St. Lawrence River. In contrast to earlier findings for metals, differences in bioaccumulation among the species and sexes were not significant for any organic compound. Site rankings based on tissue concentrations were compared among the four biomonitors for nine groups of organic compounds and found to be significantly correlated for ΣPCBs and ΣPAHs, and also for ΣBHC except for comparisons involving female *L.r. radiata*. Ranks for Σaldrin, ΣDDT and ΣCBs agreed between male and female *E. complanata* only, and there was no agreement for Σchlordane, Σendosulfan or mirex. Based on female *E. complanata*, PCBs, PAHs and CBs had similar spatial distributions, whereas the pesticides generally autocorrelated and were best represented by ΣBHC. Mussels upstream of known PCB sources contained only 66-74 PCB congeners and 14-17% mono- to tetrachlorobiphenyls vs 76-81 congeners and 30-33% mono- to tetrachlorobiphenyls in mussels downstream. As trends were more consistent for *E. complanata*, this species would be recommended for biomonitoring.

TOWARDS THE DEVELOPMENT OF A SITE-SPECIFIC WATER QUALITY OBJECTIVE FOR ATRAZINE IN THE YAMASKA RIVER, QUEBEC FOR THE PROTECTION OF AQUATIC LIFE

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The biological effects of xenobiotics in aquatic environments are often a function of the water quality in these systems. The aquatic green alga *Selenastrum capricornutum* was exposed to 11 concentrations of the herbicide atrazine in the range 0-881 µg/L. The bathing media were water collected from the Yamaska River Lac Brome (control) site in the spring and the fall. Algae incubated in fall water displayed a significant increase in the intensity of fluorescence as compared to those incubating in spring water. This effect (Kautsky effect) was observed for six intermediate atrazine concentrations which are representative of those found in the field. *In situ* investigations were also performed on the water quality of four Yamaska River sites to measure both biotic and abiotic (physico-chemical) parameters. Of the 35 water quality parameters analysed, 32 differed significantly spatio-temporally. Of these, only the nitrates (max. 4.02 mg/L) and atrazine levels (max. 40 µg/L) exceeded the water quality guideline for the protection of aquatic life (0.06 mg/L set for nitrite; 2 µg/L for atrazine). Two Yamaska tributaries experienced a reduction in populations of chlorophytic algae immediately following the atrazine application period in early June. These investigations show that herbicide spring "pulse" in river systems may alter the normal succession of phytoplankton communities. Local environmental biotic and abiotic variables are amenable for atrazine toxicity in the Yamaska River. This justifies further research towards the establishment of a site-specific objective for atrazine in this river basin.

FIELD STUDIES ON TOXICITY OF ASPEN WOOD LEACHATE TO AQUATIC LIFE

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Wood from trembling aspen (*Populus tremuloides* Michx.) is being used increasingly in the boreal forest region for pulp and paper production, as well as for building materials and special wood products. In northern British Columbia, a dark, watery, toxic leachate has been observed around woodpiles of aspen cut in winter. A study was undertaken by B.C. Ministry of Environment to measure the production of leachate from whole aspen logs stacked in the field as they would be at an industrial log storage site. The field study complements a preliminary laboratory study which described the chemistry and toxicity of leachate produced artificially from wood chips.

A standard truckload (about 18 m^3) of harvestable aspen logs, cut into 2.6-m lengths with bark on, was stacked in an open field in November 1991 and left exposed to the weather for 2 yr. The log pile began producing leachate in <4 mo and continued to do so for the duration of the study. An average of 250 L of leachate was produced on each collection following rain storms or snow melt. Toxicity of the leachate, assayed using acute tests with rainbow trout, *Daphnia*, and luminescent bacteria (Microtox), varied from weak (mean EC_{50} or LC_{50} >10%) to very strong (mean EC_{50} or LC_{50} <1%). Leachate was characterized by dark colour, acidic pH (5.0-6.5), elevated conductivity, high to extremely high BOD (to 5000 mg/L) and total organic carbon, variable levels of phenolic compounds and low dissolved oxygen.

There was no discernable effect of freezing of the wood in winter on leachate quality, and no evidence that spring thaw produced more, or more potent, leachate than summer rain. Heavier precipitation tended to produce a slightly more dilute leachate which was correspondingly less toxic. However, loads of chemical constituents or toxicity (concentrations or toxic units times volume) contained in aspen leachate remained nearly constant over the duration of the study. The lower toxicity and chemical concentrations in later samples were mostly a result of higher rainfall in the second summer producing greater volumes of more dilute leachate. There is no evidence that the release of soluble material from the wood was slowing after 2 yr in the field. Only about 3-5% of the total mass of leachable material in the aspen logs was removed during 2 yr exposure.

SURVEY OF CONTAMINANTS IN SUSPENDED SEDIMENTS AND WATER FROM THE FRASER RIVER BASIN - SPATIAL AND TEMPORAL TRENDS

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Concentrations of trace organic contaminants were measured in suspended sediments samples collected upstream and downstream of six pulp mills located in the Fraser River Basin. Sampling was conducted over three consecutive years (1992-1994) under fall low flow and winter base flow conditions. Results indicate that [1] dioxins, furans, chlorophenolics, resin acids and fatty acids measured in suspended sediments were found in higher concentrations downstream of pulp and paper mills than at reference sites upstream of the mills, [2] the same contaminants were generally found in higher concentrations during winter base flow periods compared to fall flow conditions and [3] concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF in suspended sediments have decreased from levels measured in 1990 prior to implementation of pulp mill abatement measures. Contaminants measured in suspended sediments did not exceed federal and/or provincial water quality guidelines or criteria for the protection of aquatic life.

ENANTIOSELECTIVE DEGRADATION OF α-HEXACHLOROCYCLOHEXANE IN TWELVE MAJOR RIVERS OF THE NORTHWEST TERRITORIES

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Water and suspended sediment samples were collected at 12 major rivers in the Northwest Territories to investigate the distribution of α-hexachlorocyclohexane (α-HCH) and the enantioselective degradation of α-HCH in Arctic rivers. Three stations on the Mackenzie River and one station near the mouth of 11 other northern rivers were selected for sampling. Average concentrations of α-HCH and γ-HCH in water samples collected in the central and eastern Arctic were 0.9±0.4 and 0.04±0.03 ng/L. These values are similar to the values reported for water samples collected at Amituk Lake on Cornwallis Island during the same year. The Mackenzie River is distinctively different than the other 11 rivers. The average concentrations of α-HCH and γ-HCH in water samples collected on the Mackenzie River were 0.24±0.07 and 0.13±0.06 ng/L. The two enantiomers of α-HCH were separated by gas chromatography on permethylated cyclodextrin capillary columns. The enantiomeric ratio (ER=(+)α-HCH/(-)α-HCH) for an α-HCH standard was 0.96±0.01 which is in agreement with a theoretical ER=1.00 for unmetabolized α-HCH. The average ER for water samples collected in the central and eastern Arctic was 1.01±0.04, while the average ER for water samples collected on the Mackenzie River was 0.57±0.01.

BIOASSAY: ECOLOGICAL RISK ASSESSMENT
SESSION CHAIR: P. RIEBEL

HOW ARE TOXICITY TEST DATA USED IN ECOLOGICAL RISK ASSESSMENT?

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Abstract

Ecological risk assessment (ERA) is the process of evaluating the impacts of chemical, physical and biological stressors on non-human biota. One application of ERA is in the evaluation of contaminated sites, in order to determine whether remediation or abatement measures are required. Toxicity testing is an important tool in ecological risk assessment. While field biological surveys (e.g., fish or benthic community surveys) can provide real-world evidence of impacts to species, the cause of any observed impacts cannot be determined. Toxicity testing may identify whether it is the contaminated medium that is toxic. Chemical analysis may then help to reveal which specific contaminants are responsible for the toxicity. Communication between the risk assessor and the ecotoxicologist is crucial. The risk assessor must understand how much confidence can be placed in the test data relative to the other site data (e.g., chemical analyses, field surveys), in order to determine how much weight can be assigned to these data in the weight-of-evidence approach to risk characterization. Factors influencing the risk assessor's confidence in the test data include test species and life stage sensitivity to site contaminants, test duration, and toxicity endpoint (e.g., lethality, reproduction).

Introduction

Ecological Risk Assessment (ERA) is the process of evaluating the impacts of chemical, physical and biological stressors on non-human biota. For the purposes of this discussion, ERA of chemicals stressors will be emphasized, since this is the area where toxicity test data are most relevant. An ERA can be prospective or retrospective; that is, an ERA may be used to determine future impacts of a proposed activity, such as operation of an incinerator, or to evaluate sites that are already contaminated. Toxicity tests are especially useful for this latter type of ERA.

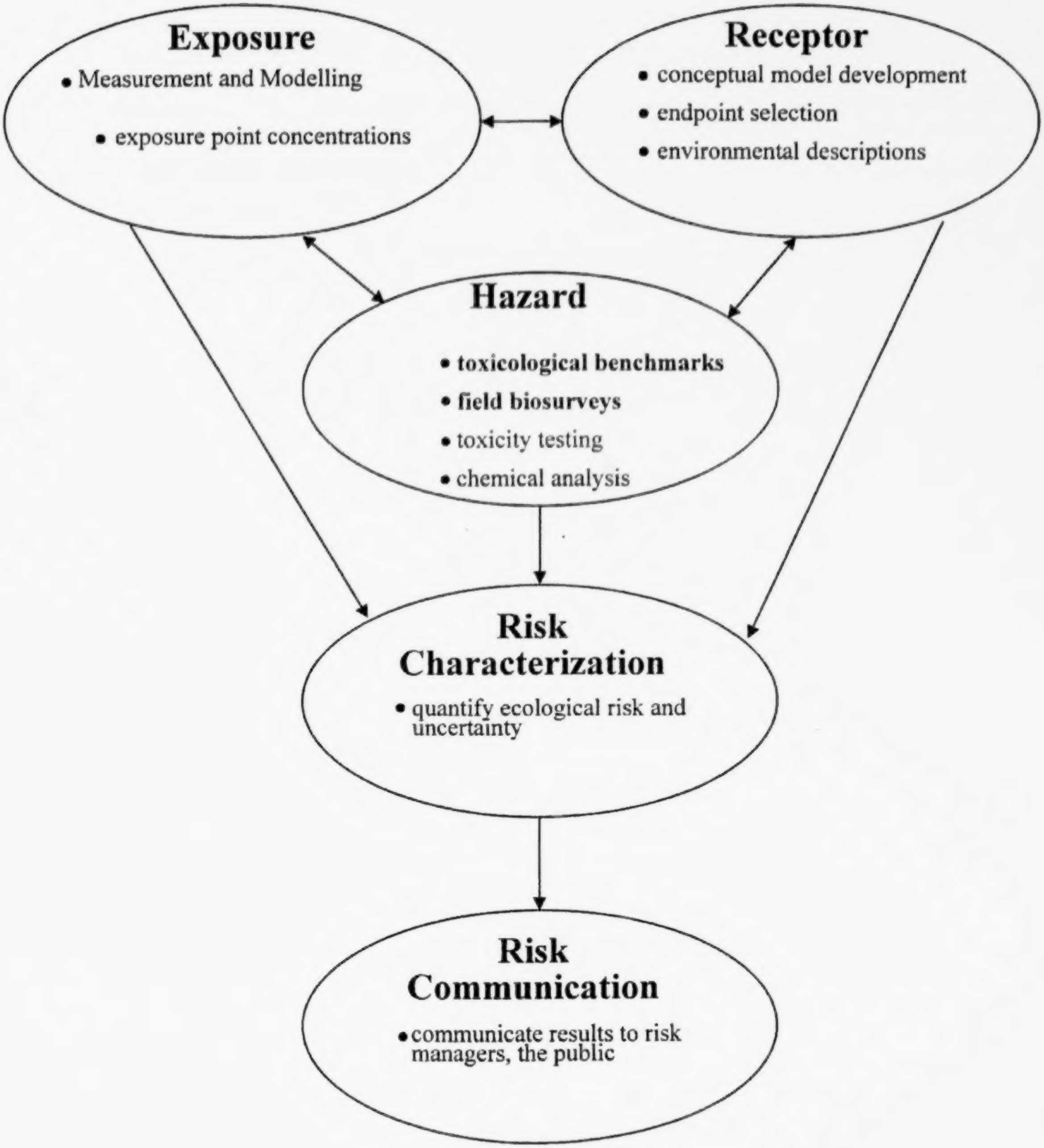
Applications of ERA

ERA can be implemented for a number of different applications. ERAs can be conducted for due diligence purposes. This is especially important in the case of property transfer transactions to determine potential liabilities to the owner of a property. Another use is for the determination of whether contaminated-site remediation is necessary. In Canada, Quebec and British Columbia have included ERA in their site cleanup guidelines, and Ontario has included ERA in the proposed cleanup guidelines. In any case, the ERA can be used in several contexts to determine the extent of remediation necessary, specifically by setting goals for: allowable chemical concentrations; and, volume of contaminated material to be remediated at a site. Another application of ERA is to evaluate remedial alternatives for their ability to reduce risk to acceptable levels.

Framework for ERA in Canada

The Canadian framework for ecological risk assessment (CCME, 1994) is illustrated in Figure 1. Toxicity testing, as is evident in this framework, is a component of hazard assessment. The hazard assessment phase involves the presentation of the data to be used in the risk characterization. These data may include field biological surveys (e.g., fish community, benthic community, small mammals, threatened or endangered species surveys), toxicity test results (e.g., rainbow trout, *Ceriodaphnia dubia*, earthworm), and chemical

Canadian Framework for Ecological Risk Assessment (modified from CCME, 1994)


Exposure
• Measurement and Modelling
• exposure point concentrations
Receptor
• conceptual model development
• endpoint selection
• environmental descriptions
Hazard
• toxicological benchmarks
• field biosurveys
• toxicity testing
• chemical analysis
Risk Characterization
• quantify ecological risk and uncertainty
Risk Communication
• communicate results to risk managers, the public

analysis of the media (water, soil, sediment, etc.) at the site. In the risk characterization phase, the various data are interpreted in order to quantify risks. Toxicity tests have two important uses in hazard assessment: (a) in the development of the toxicological benchmarks; and (b) to determine whether site media are toxic to test species under controlled conditions. These two uses are described in greater detail below.

Toxicological Benchmarks

In a hazard assessment, chemical concentrations are compared to toxicological benchmark concentrations to determine which chemicals are most likely responsible for impacts observed at the site. At present, there is no standard set of toxicological benchmarks, as there is for human health risk assessment in the United States (U.S. EPA's Integrated Risk Information System (IRIS) and Health Effects Assessment Summary Tables (HEAST) databases). As a result, the toxicological benchmarks may be regulatory values, such as water quality guidelines and sediment quality criteria (both of which are at least partially derived from toxicity tests). Moreover, these benchmarks may be derived from the primary literature. They are generally derived using Lowest-Observed-Effect-Concentrations (LOECs), an endpoint derived from toxicity tests.

The toxicological benchmarks are used in the risk characterization phase to calculate a hazard quotient, in the following way:

$$\text{Hazard Quotient} = \frac{\text{Chemical Concentration to which Organism is Exposed}}{\text{Toxicological Benchmark Concentration}}$$

A hazard quotient < 1 is generally considered acceptable, whereas a hazard quotient ≥ 1 may result in unacceptable risks.

Evaluation of Contaminated Site Media

In an ERA which is conducted to evaluate a contaminated site, toxicity tests with site media (i.e., water, sediment, soil) may determine whether the medium is toxic to test organisms under controlled conditions. This is done specifically by comparing effects of site media with laboratory controls (to ensure that the test organisms were healthy) and with reference site media (to ensure that the reference (field) site is appropriate). One of the major challenges for the ecological risk assessor involves relating toxicity test organisms (e.g., rainbow trout, fathead minnow) to resident species at the site (e.g., salmon). There are a number of factors to consider in this evaluation, including: test sensitivity;
organism ecology and behaviour (e.g., feeding, migration); physical and chemical parameters in the site environment (e.g., pH, water hardness).

The "Weight-of-Evidence" Approach

The "Weight of Evidence" Approach is one method for interpreting results in the risk characterization phase of an ERA. Field biological survey data are evaluated in order to determine whether the community looks "healthy" relative to reference site communities (i.e., a site upstream or a nearby stream with similar physical characteristics). For example, the presence of species which are sensitive to known site contaminants may indicate that chemical concentrations are not adversely affecting the community. Conversely, the presence of pollution-tolerant species and the absence of sensitive species may indicate that contaminants are affecting the community. If impacts are observed in the field, then one must deduce the cause of such impacts. These impacts may or may not be due to contaminants (e.g., physical effects such as temperature, increased predation). Therefore, we look at the toxicity test results to determine whether the contaminated site media adversely affect test organisms. We also look at which chemicals exceeded the toxicological benchmark concentrations, and to what magnitude. An adverse effect in a toxicity test and chemical concentrations which exceed benchmarks suggest that these chemicals may, in fact, be causing the impact observed in the field. However, the data are not always this simple. One may have chemicals which exceed benchmarks but no impact in the toxicity tests, and so it may be that the toxicity test, or the biological endpoint observed, is not sensitive enough to detect the impact, or something unrelated to contaminants is affecting the community. Because there are so many potential combinations of positive or negative results to the surveys, toxicity tests and comparison to benchmarks, it is important to understand how sensitive each particular test or survey is.

It is for these reasons that clear communication between the ecological risk assessor and ecotoxicologist must exist so that the risk assessor can understand how much confidence can be placed in the test data relative to other site data (e.g., field surveys, chemical analyses). Some of the factors influencing confidence in data include: test species and life stage sensitivity to site contaminants; test method reproducibility and sensitivity; test duration; and biological endpoint (e.g., lethality, growth, reproduction, behaviour).

Hierarchy of Data using the "Weight of Evidence" Approach

The general hierarchy or the "weighting" of data is such that field surveys have more weight (i.e., confidence) assigned to them than toxicity tests, and toxicity tests have more weight than chemical concentrations in site media. This is particularly true in the aquatic environment, where quantitative surveys of fish communities, fish populations, and benthic invertebrate communities are routine. For example, it is difficult to recommend remediation (e.g., dredging of sediments) if the fish and benthic communities appear "healthy". However, an impact in an aquatic community is not necessarily the result of chemicals; there could be physical influences on the community (e.g., change in temperature, number of predators, restricted habitat). Traditionally, risk assessments have generally relied on only chemical measurements at the site. This is the least reliable type of data because it ignores issues such as bioavailability and contaminant interactions (i.e., additive, synergistic effects). Also, toxicological benchmark concentrations for many contaminants would have to be modified to account for differences such as pH, water hardness, organic carbon content of sediments, since these parameters vary significantly in aquatic environments.

Aquatic toxicity tests have been routinely conducted for over 20 years. However, they are conducted using relatively few species, and the tests are conducted for a relatively short duration as compared to the length of time organisms would be exposed to contaminants in a stream. Toxicity tests do, however, give an indication of whether the contaminant mixture present at a site is toxic, and given their importance in providing this type of information, should be used in site-specific ecological risk assessments.

Reference

CCME (Canadian Council of Ministers of the Environment. 1994. A Framework for Ecological Risk Assessment at Contaminated Sites in Canada: Review and Recommendations. Scientific Series No. 199, Ottawa, Ontario.

UTILITY OF STANDARD LABORATORY TESTING IN ENVIRONMENTAL RISK ASSESSMENTS

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Effects of pure chemicals, formulations and process effluents on aquatic and terrestrial organisms are generally evaluated using laboratory testing procedures which emphasize standardization of results between laboratories and provide conditions which will elicit and detect the most sensitive response by selected surrogate organisms. These standard laboratory evaluations provide information pertaining to the acute, subchronic and chronic toxicity of a test material, through a progression of tiered tests. Environmental effects data, along with the results of testing designed to determine the environmental fate of materials, are used to perform environmental risk characterizations. These characterizations (e.g. Risk Quotients) are established utilizing toxicity data, material use data, fate and transport data, as well as estimates of exposure. Based on the risk characterizations, which incorporate exposure and toxicity, levels of concern are established for chemicals and non-target organisms. An evaluation of standard laboratory testing requirements and the utility of example data sets in risk characterizations will be reviewed. Risk characterizations or environmental assessments for various categories of chemicals (pesticides, pharmaceuticals, process effluents) will be discussed with the emphasis on evaluating environmental effects and fata data and potential ecological risk associated with the registration of a pesticide.

CONSIDERATIONS RELATED TO THE USE OF TOXICITY TESTING IN CANADA'S OCEAN DISPOSAL PROGRAM

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As part of its Ocean Disposal Program, Environment Canada is proposing the use of sediment and pore-water toxicity tests to evaluate the acceptability of estuarine and marine sediments for ocean disposal. The approach combines chemical and biological test responses to provide a more complete assessment of potential sediment toxicity. Under a tiered testing approach, sediments which fail the regulated chemical limits would be subjected to toxicity testing using five different types of tests: a 10-d amphipod acute test, a bacterial bioluminescence test, an echinoid fertilization test, a 28-d bioaccumulation test and a polychaete growth test which is still in development. In the past year, the use of the first four of these tests in ocean disposal projects on Canada's east and west coasts has generated several issues which need to be addressed. Among these is the need for more guidance on the selection of reference sediments and on the selection of appropriate test species. Also, the interpretation of toxicity due to unregulated parameters such as sulfides and ammonia must be considered. Pass/fail criteria based on sound scientific rationale must be established to justify land confinement or capping of sediments, and a weight-of-evidence approach (e.g. Triad) using site-specific studies should be considered to support the results of laboratory tests. Techniques such as Ecological Risk Assessment should also be considered to predict potential biological effects at an ocean dump site.

ECOTOXICOLOGICAL MANAGEMENT TOOL FOR CONTAMINATED SEDIMENTS

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In the past few years, GREEN PLAN monies have permitted various collaborative efforts to characterize bioanalytically contaminated marine sediments. The Centre Saint-Laurent (CSL) of Environment Canada in the Quebec region has collaborated with the Atlantic region in some of these studies. As a complement, CSL, with some of its St. Lawrence Vision 2000 monies, is attempting to conceive an ecotoxicological management tool (SEEP - Sediment Ecotoxicological Effects Potential) that would integrate results from a battery of cost-efficient bioassays. The novel approach uses data from bioassays conducted on three complimentary study compartments (i.e. interstitial water/humid solid/organic extract) chosen to facilitate decision making regarding the fate of various toxic mixtures associated with contaminated sediments (i.e. water diffusible/precipitable readily available/precipitable ultimately available). Status of development of this approach is presented with tentative interpretation of bioassay data obtained from three recent studies conducted in 1993 and 1994 (Saint John in New Brunswick, Anse à Beaufils and Cap aux Meules in Quebec).

A MICROPHOTOELECTROCHEMICAL CELL USING PHOTOSYNTHETIC MEMBRANES TO DETECT PHOTOSYNTHESIS INHIBITION IN WATER EFFLUENTS BY HERBICIDES AND METAL CATIONS

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Because the photosynthetic organism are at the beginning of the food chain, we chose them to analyse the presence of toxic pollutants in the aquatic environment. A microelectrochemical cell (80 µl) is used to monitor photocurrents produced by isolated photosynthetic membranes. In this cell, the absorbed light energy is converted in photocurrent which is related to the photosynthetic activity. We measured the inhibition of the photocurrent by various products: herbicides, insecticides, metals cations and even water samples from industry. Presently we use thylakoid extracts from two species of plants, spinach and barley, but we could work with photo system particles or intact algae. The thylakoids could be immobilized in an albumin-glutaraldehyde cross-linked matrix, that stabilized the activity of the membranes at room temperature. Thus the immobilized thylakoid membranes can be used in the field to determine the effect of water pollutants. The potential of this cell, using either free or immobilized preparations, for the rapid and sensitive measurement of toxic metal cations and herbicides in water will be discussed.

ENVIRONMENTAL ASSESSMENT OF THE LEACHATE FROM CREOSOTE-TREATED PILINGS IN SEAWATER

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[2]Koppers Industries, Inc., Pittsburgh, PA;
[3]Springborn Laboratories, Wareham, MA; [4]GERG, Texas A&M University, TX

A multiple-stage methodology utilizing a capillary column gas chromatography with mass spectrometer detector was used for the identification and quantification of polycyclic aromatic hydrocarbons (PAHs) in neat creosote material and environmental samples. Comparison of the retention times of 46 compounds contained in a neat mixture enabled the quantification of these constituents in surface water sheen, water column and sediment samples collected at Moss Landing Harbor, Moss Landing, California. Three areas of investigation comprising the evaluation of samples were: [1] evaluation of environmental conditions around existing creosote pilings; [2] evaluation of environmental conditions around restoration of pilings; and [3] evaluation of piling impact 1 yr after pile-driving. The surface sheen and water column samples were evaluated using the short-term chronic exposures with *Mysidopsis bahia*. The biological and analytical results of the field exposures are being used to evaluate and determine the risk of creosote-treated pilings on pelagic organisms in the marine environment. Creosote contains a number of organic components, some of which are biologically active. The chemical composition of creosote is critical to its fate and effects in the environment. Due to differential partitioning of these components, however, estimates of the environmental impact of creosote and creosote-treated wood cannot be solely based on the composition of creosote.

TECHNICAL EVALUATION OF THE PROPOSED DUCKWEED (*Lemna minor*) TOXICITY TEST METHOD

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Abstract

The Environmental Effects Monitoring (EEM) Program for Canadian pulp and paper mills stipulates the use of several species from different trophic levels as test organisms for the evaluation of toxicity of effluents discharged to receiving waters. One of two freshwater plant species used for the program is the common duckweed, *Lemna minor* and the method to be used is the Proposed Duckweed (*Lema minor*) Test as per AHA (1992; #8211). Toxicity testing using the duckweed may be influenced by a variety of different test conditions, such as: light intensity, light regime (photoperiod), enumeration technique for frond production, and evaporation of test solutions. An evaluation study of the proposed method will be presented, as well as test sensitivity comparisons with other sublethal tests conducted on the same effluent samples, and the use of various test endpoints for reporting.

Introduction

The Environmental Effects Monitoring Program (ECM) for the Pulp and Paper Sector requires increased biomonitoring of mill effluents discharged to the receiving aquatic environment (EC/DFO 1992). In order to protect aquatic plants, either the unicellular green alga, *Selenastrum capricornutum*, or the small aquatic macrophyte, *Lema minor*, must be used as test species (EC/DFO 1992). To date, the latter test has not been incorporated into any other aquatic toxicity test batteries for regulatory or monitoring purposes.

Generally, most pulp and paper mill effluents tested are turbid and coloured; these characteristics make it difficult to conduct the toxicity test with such samples using the green alga, *Selenastrum capricornutum* (Environment Canada 1992). Based on this assumption, and other logistical and cost considerations, many commercial biological laboratories have opted to choose the Lesser Duckweed (*Lema minor*) as a test species.

This presentation discusses a technical evaluation conducted by BEAK's Ecotoxicity Laboratory of the Proposed Duckweed Toxicity Testing method as published by the American Public Health Association (AHA 1992).

The Test Organism

Lema minor, commonly known as Lesser Duckweed (or duck's meat or water lentils) is a colonial plant from the family Lemnaceae. It is widely distributed in ponds, lakes and stagnant waters throughout North America, except the extreme north and in the Bahamas (Britton and Brown 1970). It is also found in waters in Europe, Asia, Africa and Australia. Its ubiquitous nature makes it attractive as a test species, since it can be easily collected and cultured throughout the world (Britton and Brown 1970).

Lema minor has an obovate or subcircular thallus 2.5 cm to 5 cm long, thickish, provided with stomata, obscurely 3-nerved, very rarely 4-5 nerved. The rootcaps are obtuse or subtruncate. The plant is colonial and reproduces either sexually or asexually. The fruit of the plant is symmetrical, subturbinate and wingless. The seed has a prominent hilium, deeply and unequally ribbed (Britton and Brown 1970). Duckweed is a food source for waterfowl and small animals and provides shelter for aquatic invertebrates and fish.

The Proposed Test Method

The proposed AHA test method (AHA 1992) describes a toxicity test procedure with *Lema minor*. It is described as being useful, especially for testing the air-water interface where surface-active substances, such as oil, grease and organic compounds may be concentrated. The method is described as simple, sensitive and cost-effective.

Since the method was published, no amendments have been issued. Therefore, one would assume that the test procedure has been well described, standardized and validated prior to its publication. Moreover, it is assumed that test reproducibility and repeatability have also been addressed. This is not the case with regard to this method.

Commercial toxicity testing laboratories (especially those providing sublethal tests regulated under the *Canadian Environmental Protection Act* and the *Fisheries Act*) must apply tests with a tacit presumption that they are uniform and, if necessary, that uniformity can be demonstrated. Therefore, ecotoxicological tests that are used for regulatory purposes must give the same results in different laboratories and in the same laboratory performed by different technical personnel. These concepts are referred to as repeatability and reproducibility, respectively. Of course, when tests are performed on the same sample in different places by different technicians, they are unlikely to produce identical results, since the measuring "device" is biological. In addition, observer error is also a factor.

In order to satisfy the above-mentioned requirements, biological test methods should be standardized and validated prior to their application in testing laboratories (Van Steertegem and Persoone 1993). BEAK's Ecotoxicity Laboratory undertook this challenge with the Duckweed test. A considerable amount of effort was invested in the evaluation of the test, quality control data development, the investigation of test reproducibility, and test sensitivity comparisons with other sublethal tests using other aquatic species.

Below we will briefly outline the test procedures as described in the published method and its application by technical staff in a commercial laboratory.

The method designates the Lesser Duckweed, *Lema minor*, as the test organism. The culturing procedures are simple and straightforward and describe culturing test plants in 15 L culture vessels at 25°C under constant cool-white fluorescent light: two optional light intensities are given (4300 lux or 2150 lux). The toxicity testing procedures may vary with different approaches. These approaches may introduce discrepancies in testing techniques. For example, either *static, static-renewal* or *continuous-flow* regimes may be used, depending on test solution stability (i.e., a *static* test should be used if there is low volatility or if high toxic metal concentration is suspected; *flow-through* or *static renewal* tests should be used if the samples are unstable). The test vessels may be constructed of either glass or plastic, but caution should be exercised that the test plants not adhere to the sides of test chamber. Different light intensities (4300 lux or 2150 lux) may be applied during the test exposure period, according to the method. Plants with or without fully grown roots or with roots cut off may be introduced to the test solutions. The same amount of nutrients must be added to all control and test samples. The test is to be conducted in quadruplicate and a negative (containing only nutrient) and positive control (chromate ion recommended) should be tested. Test results are expressed as % inhibition relative to the control and may be graphed using linear, semi-log or log-log plots. IC10, IC50 and IC90 are determined by graphical or statistical methods (no method is specified). Under the test method section, the protocol specifies that frond increase (a quantal value directly reflecting growth rate) is a seemingly reliable method. However, other methods that have been used include: chlorophyll content, biomass estimates, C_{14} uptake and root length (Taraldsen and Norberg-King 1990). Mortality alone is of limited value. Under the quality control section, test validity criteria are described. A test is not acceptable if more than 10% of control organisms die, are diseased or are stressed during the test (but these are not operationally defined). If the control sample produces less than a twofold increase in new fronds in 96 hours, the test is deemed invalid and the results are therefore unacceptable.

Test Method Evaluation

During the test method evaluation, a number of different key parameters were investigated and evaluated. These are described briefly below:

Test Vessels

- A comparison of plastic versus glass vessels was performed. Tests showed no difference in plant growth. Therefore, plastic vessels may be used for testing purposes.

- Chamber sizes were compared: 100 mL test chambers were tested against 50 mL test chambers. It was estimated that plants require certain volumes and water surface area for adequate growth; provision of larger test vessels yields improved growth and therefore validity criteria are more likely to be met.

Depth of Test Solution

- Shallow versus deep containers were used for testing, and untreated plants (with roots) and plants with roots cut off were used in testing. It was determined that it is simpler to use plants with roots and test organisms must be provided a minimum of 5 cm depth of test solution.

Light Intensity

- A light intensity comparison was also conducted at the two levels specified in the method (2150 and 4300 lux). The test comparison demonstrated that tests performed under lower light intensity (2150 lux) barely passed validity criteria, while plants tested with high light intensity (4300 lux) had a fourfold increase in growth.

Endpoint Determination

- Frond production estimates were performed on two different occasions by four different technicians each using two different techniques (light table from underneath the test vessels and light from above). Since the method describes that every protruding bud must be counted, the light table providing lighting from underneath the observation vessels should be used as the most reliable and repeatable method for frond counts.

- Consistency with the ECM reporting of the test endpoint requirements (i.e., NOEC, LOEC, TEC, IC25/IC50) with 95% confidence limits were considered versus the endpoints specified in the proposed method.

Test Standardization

- Standard Operating Procedures for the test method at BEAK's Ecotoxicity Laboratory were developed for both test organism culturing and full-scale effluent testing.

- Reference toxicant tests were performed. Control charts were developed and control limits were established.

- Comparisons of results of the duckweed test and those of other test species (i.e., fathead minnow and *Ceriodaphnia dubia*) were conducted using pulp and paper effluents before the test was offered to clients. Comparisons with toxicity test data from other sublethal ECM tests performed on the same effluent at the same time are presented in Table 1. These results demonstrate the low sensitivity of the Duckweed test with pulp and paper effluent in comparison with sublethal fish and invertebrate tests required by the ECM Regulatory Program.

Recommendations and Conclusions

1. Standardization and further development of the test method is required (Van Steertegem and Persoone 1993). Specific points to consider are:

    * Determination of the test precision is recommended.
    * A selection of reference toxicants for quality control requirements should be provided.
    * Determination of test organism sensitivity to various toxicants and complex effluents in comparison with other test species should be conducted.
    * Determination of the test's usefulness and application to regulatory purposes should be closely evaluated.

2. Standard development of the test under the "Biological Test Method" Publication Series should be undertaken and a new, standardized test method adaptation at the national/international level in a regulatory context should also be considered.

3. Interlaboratory evaluation ("round-robin") testing of the standardized test method should be conducted. This exercise would help to clarify the precision of the test, and will promote its use in the scientific community.

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Table 1: Comparison of Sublethal Toxicity Endpoints for Pulp and Paper Effluents using Fathead Minnow, *Ceriodaphnia dubia*, and Lesser Duckweed

Sample	Fathead Minnow			*Ceriodaphnia dubia*			Lesser Duckweed		
	IC25	IC50	TEC	IC25	IC50	TEC	IC25	IC50	TEC
Mill 1	11.4	19.5	4.4	4.3	8.7	4.4	>100	>100	>100
	0.5	1.0	<1.56	2.9	5.2	<1.56	38.5	86.7	35.4
	1.8	24.1	1.7	3.4	7.7	1.7	43.5	>100	35.4
Mill 2	>100	>100	>100	41.3	70.5	70.7	>100	>100	35.4
Mill 3	16.0	24.7	8.8	0.6	1.3	<1.56	>100	>100	>100
	23.7	58.2	17.3	3.3	8.3	5.5	>100	>100	>100
Mill 4	23.3	>100	8.8	14.5	22.5	8.8	>100	>100	>100
	48.3	>100	35.4	22.3	46.6	35.4	>100	>100	>100
Mill 5	8.0	16.3	2.2	0.6	1.2	<1.56	>100	>100	>100
	8.4	19.0	5.5	0.63	2.86	0.55	>100	>100	>100
Mill 6	9.5	15.8	<6.25	5.8	10.3	4.4	>100	>100	>100
Mill 7	1.1	2.0	<0.78	<0.39	<0.39	<0.39	>100	>100	>100
	1.0	1.7	0.6	0.08	0.2	0.14	>100	>100	70.7
Mill 8	3.7	5.7	2.2	1.0	2.2	1.1	>100	>100	>100
	1.28	2.37	1.1	1.86	2.7	2.21	>100	>100	70.7
Mill 9	32.6	54.9	17.7	1.6	2.5	<1.45	72.0	>100	8.8
	26.1	42.7	17.7	2.8	4.2	4.4	61.7	>100	70.7
Mill 10	11.5	18.0	8.8	19.5	30.2	35.4	>100	>100	>100
	18.2	29.7	17.7	30.3	44.2	17.7	>100	>100	>100
	15.0	23.6	8.8	17.9	28.7	17.7	>100	>100	70.7

THE PHYSIOLOGICAL BASIS OF PROTECTIVE EFFECTS OF DISSOLVED ORGANIC CARBON AGAINST COPPER AND CADMIUM TOXICITY TO RAINBOW TROUT

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Although the protective effects of dissolved organic carbon (DOC) against metal toxicity to fish are well established, the physiological basis of its protection is not known. In addition, possible adverse effects of DOC itself on fish have not been investigated. We were able to prepare enough of a concentrated DOC solution from a marsh to run 5 d flow through experiments using adult rainbow trout (*Oncorhynchus mykiss*, ~200 g) fitted with dorsal aortic cannulae. Fish were exposed to synthetic soft water alone, soft water plus 5 mg C/L DOC, soft water plus a Cu (0.3 µM) and Cd (0.06 µM) mixture, and Cu and Cd plus DOC. Aortic cannulae allowed repetitive blood sampling for determination of respiratory and ionoregulatory status of the fish during the exposures. Our results show that 5 mg C/L DOC alone has no effect on trout blood gas status ($P\mathrm{O}_2$, $P\mathrm{CO}_2$), blood pH, and plasma ion concentrations. There was some indication that DOC reduced the "stress" of the soft water exposure, as seen by lower blood lactate and plasma glucose concentrations. In the metals plus DOC experiments, results to date indicate that 5 mg C/L DOC slows the entry of Cd into fish blood, but doesn't slow Cu entry, and DOC reduced stress of the metal exposures on the fish. Overall, low concentrations of DOC have only beneficial effects on trout, but physiological effects of higher concentrations of DOC still need to be investigated.

TOXICITY IDENTIFICATION/REDUCTION EVALUATION - INDUSTRIAL APPLICATIONS
SESSION CO-CHAIRS: E.G. BADDALOO and D.A. BIRKHOLZ

MODIFIED TOXICITY IDENTIFICATION EVALUATION STUDIES FOR ACHIEVING MINING SECTOR MISA COMPLIANCE

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Results of initial MISA toxicity compliance monitoring for a multiple effluent stream mining operation indicated the presence of sporadic acute toxicity. Traditionally, only small scale acute and sub-lethal species (i.e. *Daphnia magna*, *Ceriodaphnia dubia*, *Pimephales promelas*, Microtox) have been utilized during Toxicity Identification Evaluation (TIE) studies. These methods had proven to be very expensive and of limited value in planning the future direction of mining effluent treatment. A more direct and economical approach to toxicity investigations was needed to prepare for the 1997 compliance deadline for non-lethality and water chemistry objectives. A modified EPA - TIE investigation was initiated on the problem effluent streams. Phase I modifications were made to include both MISA compliance organisms, *D. magna* and rainbow trout (*Oncorhynchus mykiss*). Phases II and III were replaced with effluent treatability assays derived from toxicity reduction/elimination information obtained during Phase I procedures. Information on potential toxicant speciation under the various treatment conditions was also collected. Preliminary results indicate that variations in the applied treatment, as well as the degree of treatment will be required for the different effluent streams to obtain non-acutely toxic effluent. Ongoing laboratory tests are being conducted to achieve consistency and confidence in the results, allowing plant operators to make informed decisions regarding the (expensive) changes to be made in their effluent treatment facilities over the next few years.

DEVELOPMENT OF THE WATER CAPPING OPTION FOR RECLAMATION OF OIL SANDS FINE TAILS

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In the extraction of bitumen from oil sands at Syncrude's plant in northeastern Alberta, large quantities of fluid tailings are produced. Under present operating conditions, the extraction tailings segregate: coarse solids to form a beach, while a fluid fraction further separates into water and fine tails zones. About 0.1-0.2 m^3 of a mature fine tails (MFT) per t of ore processed. MFT is a slowly densifying aqueous suspension of fines (>35 wt%) and unrecovered hydrocarbons (2 wt%). Up to one billion cubic metres of MFT are predicted to need reclamation when operations cease. The water associated with the fine tails is acutely toxic to aquatic organisms and cannot be directly released into a receiving environment. Because of the large volumes involved, an integrated approach to reclamation of the MFT is being developed. One of the options involves retaining the fluid character of the MFT and its incorporation into a lake system with a water cap layer overlying the MFT. Because of the rheological properties of MFT and the optimization of water capping depths, the potential for mixing of layers is small. Ongoing research programs have shown that a viable lake environment will be sustained in the water capping layer and the concept is technically feasible. Water capping depends on the long term containment of MFT into geotechnically stable areas (mined out pits), the isolation of the MFT and its release water from direct contact with the surrounding environment, and the establishment of a productive and stable aquatic environment in the capping layer. In this presentation, a summary of the field and laboratory studies will be given. Various physical, biological, toxicological and chemical aspects of the water capping approach will be described and directions for future research will be highlighted. The development of a stable, productive and self-sustaining lake ecosystem in the water capping

layer in contact with MFT appears to be a viable method for safe and efficient reclamation of oil sands fine tails.

THE EFFECTS OF OIL SANDS WASTEWATER ON FISH RESULTING FROM EXPOSURE TO SUBLETHAL CONCENTRATIONS

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Rainbow trout, *Oncorhynchus mykiss*, were exposed to sublethal concentrations of oil sands wastewater in flow through laboratory experiments as well as to artificial ponds containing sublethal concentrations of tailings pond water and the fine tails in order to study the viability of the wet landscape remediation option. Large (200-300 g) fish were used for all the exposures in this preliminary study and the following data were collected: blood cell counts, sex hormone concentrations, sexual maturation, stress protein concentrations, PAH-metabolites in bile, condition factors, liver somatic indices, mixed function oxygenase induction, PAHs in muscle, external condition and the condition of internal organs. The data obtained from this study revealed no adverse effects upon fish during extended field exposures. Given similar exposure conditions in the release waters of a wet landscape reclamation, the data suggest that there may be no adverse effects upon fish; however, longer-term studies, other indicator organisms and additional chronic tests should be conducted.

COMPARISON OF ACUTE TOXICITY OF VARIOUS OIL SANDS PROCESS AND RECLAMATION WATERS

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At Syncrude Canada Ltd's Mildred Lake operation in northeastern Alberta, bitumen is separated from the oil sands using a caustic hot water flotation method. In the dispersion of the oils sands, the aqueous leachates produced are acutely toxic to aquatic organisms. The acute toxicity has been shown to be explained mainly by the low molecular weight organic acid fraction, which has been identified as the naphthenic acid group. In this presentation, results of bioassays of waters collected from various natural sites and from process affected sites throughout the Syncrude lease are reported. In addition, waters from reclamation sites were included in the study. Acute toxicity of these waters was assessed using bioassays at various trophic levels (fish, zooplankton, bacteria, algae). The properties of the waters were determined and the results of the comparison between water composition and toxicity will be presented. A strong relationship between the levels of naphthenic acid and the acute toxic responses was found. While fresh extraction tailings waters have a high acute toxicity, the same waters isolated from fresh input of tailings show a relatively rapid improvement in the toxic character and a drop in the levels of naphthenic acids. Based on ongoing research, natural bioremediation processes seem to be proceeding in process affected waters and under natural conditions will dramatically improve water quality over short periods of time. It should be possible to stimulate these natural microbial processes to optimize the rate of detoxification of oil sands process related waters and this is an important component of the reclamation options.

ISOLATION AND IDENTIFICATION OF TOXICANTS PRESENT IN CREOSOTE-CONTAMINATED GROUNDWATER

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Groundwater, taken in the vicinity of a creosote-contaminated site was subjected to a process called Toxicity Identification Evaluation (T.I.E.). Toxicants were isolated by applying the pH adjustment solid phase extraction test. Identification of toxicants, in toxic fractions, was performed using chemical derivatization followed by GC/MS as well as LC/MS. Compound groups identified include: hydroxylated polycyclic aromatic nitrogen heterocycles (HPANH), polycyclic aromatic hydrocarbons (PAH), chlorophenols (CP), hydroxylated polycyclic aromatic hydrocarbons (HPAH), phenols (P) and polycyclic aromatic nitrogen heterocycles (PANH). Concentrations were estimated to be: HPANH > PAH > CP > HPAH > P > PANH. The identification of HPANH and HPAH suggests that these are microbial transformation products of PANH and PAH which are present in creosote.

FATE AND EFFECTS OF PAHs IN THE AQUATIC ENVIRONMENT
SESSION CO-CHAIRS: J. HELLOU and J.L. METCALFE-SMITH

POLYCYCLIC AROMATIC HYDROCARBON COMPOSITION AND POTENTIAL SOURCES FOR SEDIMENT SAMPLES FROM THE BEAUFORT AND BARENTS SEAS.

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Introduction

Coastal zones adjacent to industrialized regions have significant, obvious elevations in surficial sediment PAH concentrations as a direct consequence of human activities. Assessment of anthropogenic influence becomes more difficult in remote areas because PAHs from anthropogenic sources (fossil fuel discharges and combustion) can be masked by PAHs from natural sources (oil seeps, bitumens, forest fires). The Arctic provides the prospect of a less complex regime since the most important source of anthropogenic PAHs is expected to be the long range transport of combustion emissions (e.g. Patton et al. 1991). Here we use principal components analysis (PCA) modeling as a tool for comparison of PAH distributions between the Beaufort Sea and the Barents Sea and for interpretation of PAH distributions in terms of potential sources and their geochemistry.

Results and Discussion

PAH analyses of sediments incorporated a full range of perdeuterated PAH surrogate standards (Yunker et al. 1993, and ref. therein). The parent PAH profiles that are shown for each region in Fig. 1 include naphthalene (Na), fluorene (Fl), and molecular-mass totals for phenanthrene and anthracene (178), fluoranthene and pyrene (202), benz[*a*]anthracene, chrysene and triphenylene (228), benzo[*b*/*j*/*k*]fluoranthene, benzo[*e*]pyrene and benzo(a)pyrene (252), indeno[1,2,3-cd]pyrene and benzo[*ghi*]perylene (276) and dibenz[*a*,*h*]anthracene (278). The black (left) bars at 202, 252 and 276 indicate the concentrations of fluoranthene, benzo[*b*/*j*/*k*]fluoranthene and indeno[1,2,3-*cd*]pyrene respectively and the grey (right) bars indicate pyrene, benzo[*e*]pyrene and benzo[*ghi*]perylene. For PCA, data were mid-range normalized, log transformed and mean centered (Yunker et al. 1994). Normalization also permitted PCA comparison to potential sources (e.g. oil and atmospheric particulate) and to sediments and soils from other studies. PAH variables shown in Fig. 2 are phenanthrene (Pn), fluoranthene (Fl), pyrene (Py), benz[*a*]anthracene (BaA), chrysene and triphenylene (Chr), benzo(*b*/*j*/*k*)fluoranthene (BFl), benzo[*e*]pyrene (BeP), benzo[*a*]pyrene (BaP), indeno[1,2,3-*cd*]pyrene (IP) and benzo[*ghi*]perylene (Bghi).

Parent PAH concentrations are significantly higher in samples of suspended particulate and sediment from the Mackenzie River and Mackenzie shelf of the Beaufort Sea than in sediment from the Barents Sea (Fig. 1). Concentrations exhibit little change between the Mackenzie River and shelf, but decrease slightly at the shelf edge. Barents Sea sediments have the highest concentrations in the southeast (near the major rivers), are slightly lower in the north and west of the sea (near Spitsbergen), and are lowest near Novaya Zemlya. In most cases there is little change in PAH composition between surface layers and pre-1952 sediments (depth horizon of 10-20 cm based on radionuclide penetration).

Beaufort Sea PAH profiles and PCA projections indicate higher proportions of phenanthrene, benzo[*e*]pyrene and benzo[*ghi*]perylene than samples from other locations (Figs. 1 and 2), and manifest low anthropogenic inputs relative to the natural background (Yunker et al. 1993). Fluoranthene is less than pyrene, indeno[1,2,3-*cd*]pyrene less than benzo[*ghi*]perylene and, in all except one sample, benzo[*b/j/k*]fluoranthene less than benzo[*e*]pyrene. In the Barents Sea the concentration of benzo[*e*]pyrene is universally lower than benzo[*b/j/k*]fluoranthene (Fig. 1). The major molecular-mass constituent is usually 252, and this is normally followed by 276 and then 202. Relative proportions of the four PAHs which comprise the 202 and 276 molecular-mass totals vary in different regions of the Barents Sea, and serve to classify samples into three groups. PCA projections support the classification and indicate a much larger variation in PAH composition for samples from the Barents Sea than from the Beaufort Sea (Fig. 2).

Pyrene and benzo[*ghi*]perylene predominate over respectively fluoranthene and indeno[1,2,3-*cd*]pyrene in samples from the west and north sides of the Barents Sea. Here the distributions of the alkyl naphthalenes and phenanthrenes and the prevalence of petrogenic isoprenoids are consistent with a petroleum source. Fluoranthene and indeno[1,2,3-*cd*]pyrene predominate in samples from the south and east sides of the Barents Sea. Samples collected from the west and north sides of Novaya Zemlya generally have a combustion alkyl PAH distribution for the naphthalenes and phenanthrenes and low amounts of the alkyl PAHs. Fluoranthene predominates in most samples and molecular-mass 202 is always an important constituent.

Typical oils from the Mackenzie River and Beaufort Sea have much higher proportions of phenanthrene than sediment samples and have very different PCA projections (Fig. 2). Arctic atmospheric aerosols (Patton et al. 1991) are most similar to the Barents Sea sediments in their PAH molecular-mass ratios and PCA projections. Samples obtained close to Novaya Zemlya project close to surficial sediments from the Mediterranean and to soils from an industrialized area in England (circled area in Fig. 2; Jones et al. 1989, Lipiatou and Saliot 1991). The obvious inference is that these sediments contain combustion input from industrialized areas in eastern Europe. However, surface sediments from northern Novaya Zemlya have elevated ^{239,240}Pu and a contribution from bomb-derived combustion is also possible. PCA projections for samples from the southeast Barents Sea partially overlap projections for surficial sediments from remote regions of North America (circled in Fig. 2; Tan and Heit 1981, Baker et al. 1991). The presence of high proportions of indeno[1,2,3-*cd*]pyrene in these samples suggests wood soot and coal ash as a primary source (Figs. 1 and 2).

Conclusions

PCA clearly distinguishes PAH sources for the Arctic; anthropogenic inputs are important in the Barents Sea whereas natural inputs are important to the Beaufort Sea. The uniformity and the relatively high concentration of Beaufort Sea PAHs reflects a river dominated input of natural PAHs which tends to overwhelm any anthropogenic sources. Despite the greater anthropogenic influence in the Barents Sea, PAH concentrations in Barents Sea sediments are generally 2 to 20 times lower than in Beaufort Sea sediments.

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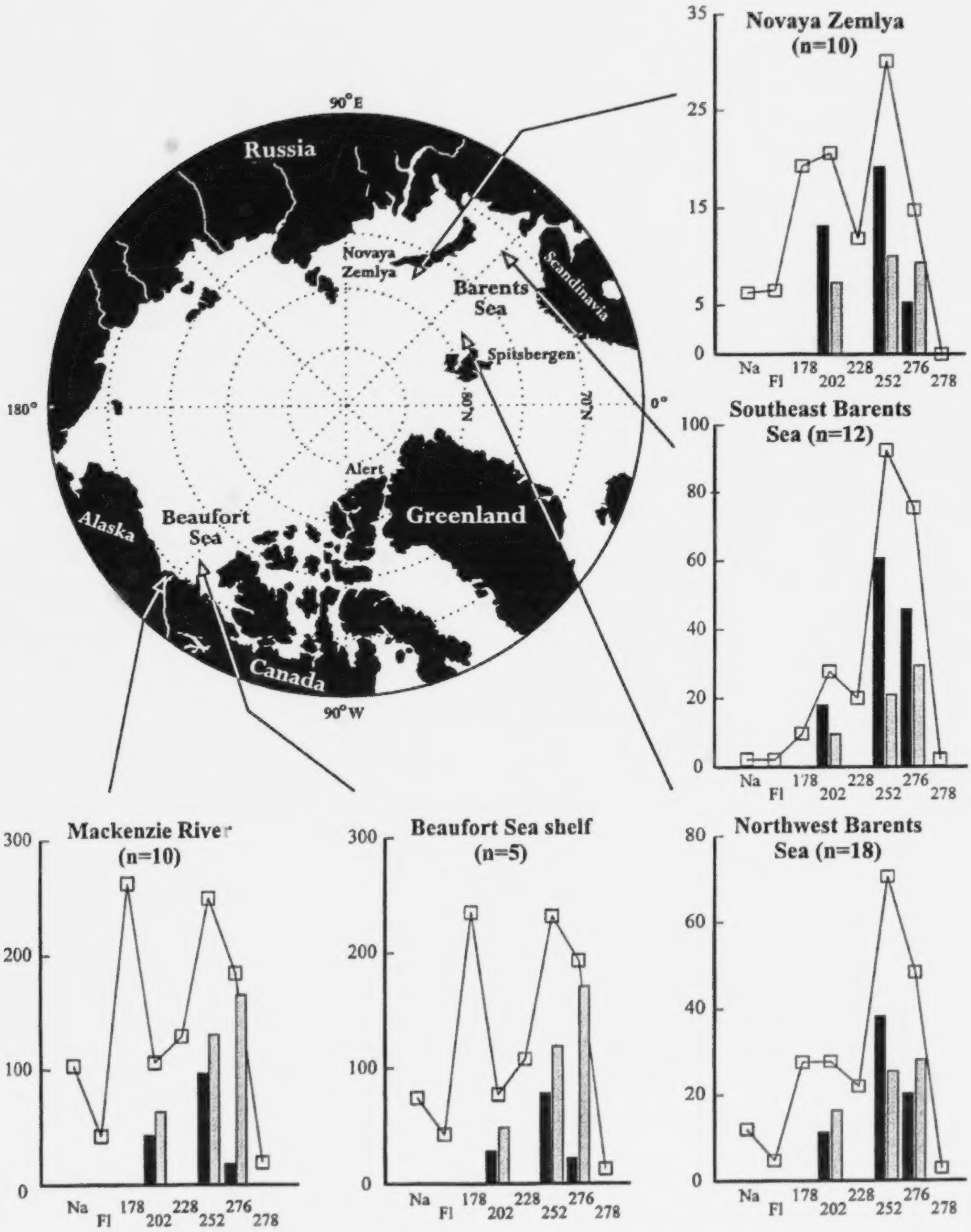

Fig. 1. PAH concentrations (ng/g) and profiles for the Beaufort and Barents Seas.

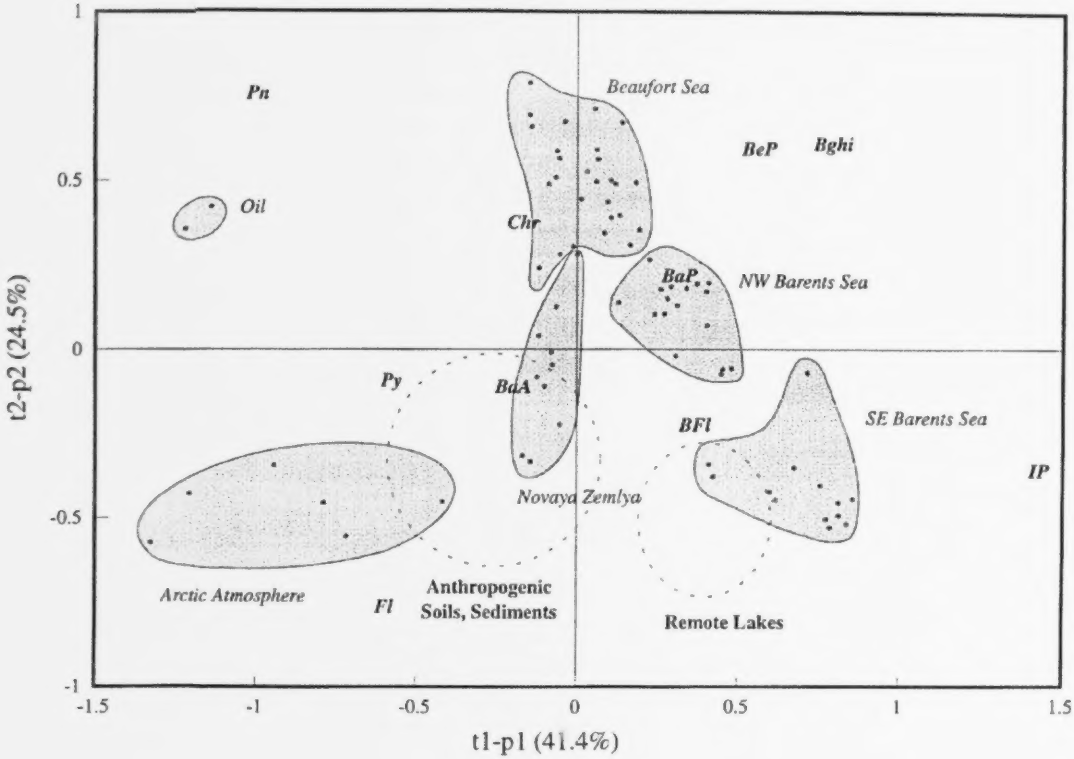

Fig. 2. Biplot of Arctic samples with parent PAH variables.

INTERPRETATION OF ALKANE AND PAH DATA FROM RECENT SEDIMENTS COLLECTED IN THE STRAIT OF GEORGIA.

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Suspended sediment samples from the Fraser River and sediment cores from dump site, pulp mill and reference locations in the Strait of Georgia and Howe Sound have been analyzed for alkanes and parent and alkyl PAHs (polycyclic aromatic hydrocarbons). Time horizons have been determined using ^{210}Pb. Principal components analysis (PCA) provides a valuable assessment of which hydrocarbons covary in sediments and facilitates interpretation of the complex downcore profiles. With PCA the independent inputs of higher plant detritus, petroleum alkanes, petroleum PAHs, anthropogenic (principally combustion) PAHs and aromatized plant derivatives (retene, simonellite, etc.) could be identified and traced to likely sources. Separate PAH maxima that correspond to approximately the 1950s and the 1970s are a general feature of cores from the region, while more recent PAH inputs are most evident in core samples from locations close to the dump site off Vancouver. Parent PAH profiles and PCA projections for the southern B.C. samples are similar to soil and sediment samples from other industrialized areas of the world, and distinct from most samples from the Arctic.

ECOLOGICAL RISK SCREEN FOR PAHs IN SEDIMENTS NEAR TWO PRODUCED WATER DISCHARGES AT COASTAL PRODUCTION PLATFORMS IN THE GULF OF MEXICO

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Preliminary screens for risks to biota, were done on PAHs in sediments associated with produced waters from platforms at Delacroix Island and at Bay de Chene, in open bays of the Louisiana coast. Sediment samples were taken in Spring 1993 at the discharge sites, along three transects at Delacroix Island and along four transects at Bay de Chene (at intervals of 100, 300, 500 and 1000 ft), and at two reference locations for each discharge site. A screen for deleterious effects on biota was done by comparing concentrations to the Effects Range-Median (ERM) and Effects Range-Low (ERL) criteria of Long et al. 1995. Only sediment samples from the discharge site at Bay de Chene exceeded ERM concentrations for either total PAH, or individual and total high molecular weight PAHs. The ERL criteria for total and individual PAH concentrations were exceeded at, and 100 m from the discharge at Delacroix Island. At Bay de Chene the ERL criteria for total and individual PAH concentrations were exceeded at the discharge, as well as at 100 and 300 m stations.

One U.S. Department of Energy (USDOE) mission is cost-effective development of domestic oil and gas resources with proper concern for the environment. This includes the potential costs of compliance with regulations and effects on domestic oil and gas supplies. Most of the current (and projected future) oil and gas platforms in the U.S. are located in the central and western Gulf of Mexico. This area supports economically important commercial and recreational fisheries, as well as unique, socially valued ecosystems and several endangered and threatened species. Oil and gas production are often accompanied by a saline wastewater, called produced water. In offshore and coastal areas, this wastewater may be discharged to surface water. Produced water may contain a number of contaminants, including oil and grease, organic compounds, heavy metals and radionuclides. Many of these contaminants are toxic to marine organisms at high concentrations.

Potential human health and environmental impact in the Gulf of Mexico, from discharge of produced water, concern regulators at state and federal levels, environmental interest groups, industry and the public. Current regulations in the United States require or propose a zero discharge limit for coastal facilities based primarily on studies performed in low energy, poorly flushed environments. Produced water discharges in coastal Louisiana, however, include a number located in open bays, where potential impacts are likely to be larger than the minimal impacts associated with offshore discharges, but smaller than those demonstrated in low-energy canal environments.

The USDOE program in the Gulf of Mexico consists of two interactive ongoing projects. One project, "Environmental and Economic Assessment of Discharges from Gulf of Mexico Region Oil and Gas Operations" (the USDOE field study), is done by Continental Shelf Associates, Inc. and their subcontractors, one of which, Steimle & Associates, Inc. supplied the data for this report. The objectives include:

- assessment of the fate and environmental effects of contaminants in produced water;
- assessment of the catch, consumption, and human use patterns of seafood species collected from coastal and offshore waters;
- assessment of the economic effects of issued and proposed regulations on offshore oil and gas producers in the Gulf of Mexico Region.

The other project, "Produced Water Risk Assessment and Programmatic Support", was assigned to the authors at the Biomedical and Environmental Assessment Group at Brookhaven National Laboratory (BEAG/BNL). Our objectives are to:

- provide technical and programmatic support to USDOE in its research effort to characterize health and environmental impacts of produced water discharges;
- perform human health and ecological risk assessments for contaminants discharged in produced water;
- provide scientific bases for risk management actions by State and Federal agencies.

The state of Louisiana planned to allow open bay discharges to continue through January 1997. To provide technical support for this decision, BEAG/BNL did a preliminary ecological assessment of the potential impact of pre-termination data from sites (Fig. 1, Table 1) that were to be studied for recovery from produced water discharges. These sites fell within the output of 90% of the Louisiana discharges, which are low volume, less than 5,000 bbl/d (Boesch and Rabalais 1989). They are typically found in shallow water depths: Delacroix ~1.5 m; and Bay de Chene ~2.3 m.

Table 1. Open bay study sites

Site	Termination of Discharge	Environment	Discharge (bbl/day)
Delacroix Island Tank Battery #1	1 May 1993	Saline, Open-Water	1,970
Bay de Chene Tank Battery #5	1 July 1993	Saline, Open-Water	3,825

Materials and Methods

Benthos, sediment and core samples were collected at discharges, stations, and reference stations on the transects shown in Fig. 2 and 3. Methods of handling, treatment and analyses of the samples have been described elsewhere by Mullino et al. (1995).

Delacroix Island

The Delacroix Island Oil and Gas field, approximately 30 miles (48 km) southeast of New Orleans, was in constant production since 1940. Eleven wells were in production in the field at the time of pre-termination sampling (April 1993).

The field contains numerous canals and their remnants, in an area that is a subsiding delta with broken marsh, numerous small water bodies, and a few large open bays. This area is highly influenced by the Mississippi River because of the Caernarvon diversion, about 15 miles (24 km) northwest of the field. The purposes of this diversion are to bring sediment-rich Mississippi River water to the subsiding delta, and reduce saltwater intrusion.

At Delacroix Island (Fig. 2), samples were taken on three transects (NW, NE & S), with stations at the discharge 100, 300 and 500 m from the discharge, and at two reference sites on the NW and NE transects. Because of land formations only the NW and SW transects had 1000 m stations.

Bay de Chene

The Bay de Chene Oil and Gas Field lies in the Barataria Basin, approximately 42 miles (67 km) south of New Orleans. The field was in constant production since 1942, with four producing wells at the time of the pre-termination sampling (May 1993).

The sampled tank battery is located in Hackberry Bay, a large open bay that is typical of the Barataria system. The area is influenced seasonally by the Mississippi River, but to a lesser extent than the Delacroix Island Area.

At Bay de Chene (Fig. 3), the collection stations were on 4 transects (NW, NE, SW & SE), with a reference station for each of the two northern transects. Only 500 m and 100 m stations were sampled on the SE transect, because of the presence of field facilities. The 100 m station along the NE transect was an abandoned fuel dock, located in a channel.

Risk Assessment

A screening analysis was done as a preliminary assessment of the pre-termination sediment sample data to comply with the urgent needs of USDOE, related to the State of Louisiana's permits for continued operations. More-detailed analyses of the ecological risks from produced waters at these two sites are being done by others.

The screening analysis used a recent update by Long et al. (1995) of the Long and Morgan (1990) ERL and ERM criteria for potential adverse ecological effects of sediment contaminants. (ERL = effects range low, adverse effects at the 10th percentile of tests in a Biological Effects Data Base; ERM = effects range median, effects at the 50th percentile of the tests.) Sediment values of a contaminant that are less than the contaminant's ERL value were defined as a minimal effects range, where effects "...would rarely be observed" (Long et al. 1995). Concentrations at and above the ERL value, but less than the ERM value, "...represent a possible-effects range within which effects would occasionally occur". Concentrations at or above the ERM value "...represent a probable effects range within which effects would frequently occur".

This presentation concentrates on PAHs. A report on screening of naturally occurring radioactive materials, inorganic and other chemical pollutants was presented elsewhere (Meinhold et al. 1995).

Results

At Delacroix Island total and individual PAH concentrations exceeded ERL criteria at the discharge and

100 m stations (Table 2). Acenapthene exceeded the ERL Value at the 300 m and 500 m stations of the northwest transect. No criteria values were exceeded at 1000 m and reference stations, and no PAHs exceeded ERM values at any stations.

Table 2. Concentrations of PAHs in Sediment Samples (0 to 5 cm) at Delacroix Island that Exceeded ERL Criteria (Discharge values are the averages from three core samples).

Contaminant	ERL (ppb)	Measured (ppb)	Station
Total PAH	4,022	12,871	Discharge
		6,056	100 m NW
Acenaphthene	16	32	Discharge
		99	100 m NW
		180	300 m NW
		69	500 m NW
		210	100 m NE
		71	300 m NE
		140	500 m NE
Anthracene	85	200	100 m NW
Fluorene	19	68	Discharge
Naphthalene	160	173	Discharge
Benzo(a)anthracene	261	350	100 m NW
Fluoranthene	600	900	100 m NW

Table 3. Concentrations of PAHs in Sediment Samples (0 to 5 cm) at Bay de Chene that Exceeded ERM Criteria (Discharge values are the averages from three core samples).

Contaminant	ERM (ppb)	Measured (ppb)	Location
Total PAH	44,792	162,152	Discharge
Benzo(a)anthracene	1,600	12,000	Discharge
Benzo(a)pyrene	1,600	9,000	Discharge
Chrysene	2,800	11,000	Discharge
Dibenzo(a,h)anthracene	260	1,700	Discharge
Fluoranthene	5,100	8,100	Discharge
Pyrene	2,600	6,100	Discharge
High Molecular Weight PAH	9,600	47,900	Discharge

At Bay de Chene only the discharge station samples exceeded ERM criteria for total and individual PAHs (Table 3). Total and individual PAH concentrations exceeded ERL criteria at the discharge, 100 m, and 300 m stations (Table 4). High concentrations at 100 m NE probably reflect the influence of the abandoned loading dock.

Table 4. Concentrations of PAHs in Sediment Samples (0 to 5 cm) at Bay de Chene that Exceeded ERL Criteria (Discharge values are the averages from three core samples).

Contaminant	ERL (ppb)	Measured (ppb)	Location
Total PAH	4022	72685	Discharge
		5370	100 m NW
		4075	300 m NW
		11577	100 m NE
		6336	300 m NE
Acenaphthene	16	213	Discharge
		48	100 m NE
		20	300 m NE
Anthracene	85.3	573	Discharge
		86	100 m NE
Fluorene	19	320	Discharge
		22	100 m NW
		33	300 m NW
		67	100 m NE
Naphthalene	160	160	Discharge
Phenanthrene	240	1363	Discharge
		250	100 m NE
		260	300 m NE
Benzo(a)anthracene	261	4787	Discharge
		340	100 m NE
		350	300 m NE
Benzo(a)pyrene	430	3683	Discharge
Chrysene	384	4433	Discharge
		470	100 m NE
Dibenzo(a,h)anthracene	63.4	687	Discharge
		70	100 m NE
Fluoranthene	600	4300	Discharge
		910	100 m NE
		650	300 m NE
Pyrene	665	3167	Discharge
		730	100 m NE

Discussion

Before cessation of operations, PAH levels in surface sediments were generally elevated above ERL criteria, up to 100 m in radius around the two discharges. At Bay de Chene, ERL criteria were also exceeded at 300 m, particularly on the NE transect. This could reflect a combination of factors: a rate of produced water discharge that was approximately double the rate at Delacroix Island (Table 1); greater chance for distribution of the discharge because of turbulence (see discussion below); and the presence of a channel; and residual sediment contamination from the abandoned dock at the 100 m station in that channel. Nevertheless, sediment samples from the Bay de Chene discharge station were the only ones that exceeded ERM criteria for PAHs.

If sediment PAH concentrations are a determining factor, one could predict from our screening results that benthic biota would be adversely affected within a 100 m radius from open bay discharges of produced waters. The pre-termination sediment PAH concentration data were supplied to us by Steimle & Associates, Inc. These data are part of a report by Mullino et al. 1995 on the USDOE field study. They reported depressions of numbers of species (amphipod, gastropod, bivalve, and polychaete) and/or individuals at less than 100 m from the discharges. These field observations agree with other observations on open coastal bays off Louisiana (Neff et al. 1992; Rabalais et al. 1992).

The pre-termination benthic effects were greater at the Delacroix Island discharge station than at the comparable Bay de Chene station. Mullino et al. explained this as a result of hydrology of the environment. Although the Delacroix discharge was approximately half that at Bay de Chene, there was less opportunity for turbulent mixing and dilution of the discharge, because the Delacroix environment was semi-enclosed. It was suggested that the Delacroix discharge was more likely to produce a hypersaline nonoxygenated layer on the bottom, as supported by data, from the 2 sites, on the chlorinity of pore water (Mullino et al. 1995).

The effects of hypersalinity and other contaminants can't be separated from those of PAHs. Mullino et al. 1995 reported that multiple regression analyses identified sediment concentrations of dibenzothiophenes (not listed in the criteria values) at the stations as negatively correlated to numbers of individuals around both sites. Dibenzothiophenes and fluoranthenes were negatively correlated with numbers of individuals and numbers of species at Bay de Chene.

For stations beyond 100 m, predictions of detrimental effects, based on the comparisons to the criteria of Long et al. 1995, do not appear to be matched by the benthic data. Published letters (Chapman 1995a,b) indicate that there is no reason to expect such a relationship. Comparisons to standard criteria based upon biological testing, and field observations each have their own role in the analysis of ecological risk: "...numerical environmental quality criteria are useful generically for screening purposes, but not for definitive assessments of, in particular, areas of the environment that fall between 'very clean' and 'very polluted'."

Acknowledgements

This work was supported by the United States Department of Energy, Office of Fossil Energy, through the Metaire Site Office, with guidance from Dr. Brent Smith as project officer. The primary contractor for the field study described in the text was Continental Shelf Associates, Inc., Jupiter, Florida. Steimle and Associates, Inc., Metaire, Louisiana supplied the data used the analysis. Work performed under Contract No. DE-AC02-76CH00016 with the U.S. Department of Energy (A.F. Meinhold, P.I.).

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POPULATION, COMMUNITY, AND BIOINDICATOR RESPONSES TO CREOSOTE IN AQUATIC MESOCOSMS.

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This presentation discusses the objectives, approach and preliminary results of a three year study focusing on the development and validation of bioindicators that are relevant to responses at the population and community level. The study focuses on creosote, a common wood preservative derived from coal tar distillate and containing approximately 85% mixed polycyclic aromatic hydrocarbons (PAHs), as a model stressor. The first year of the study documented the effects of creosote at the population and community level in aquatic mesocosms, and provided preliminary results for the selection of several bioindicators for use in further studies. Activities during the 1994 field season focused on the establishment of the aquatic ecosystems and a refinement of the methods to be used in the treatment and sampling of experimental mesocosms. Liquid creosote was applied to the mesocosms by subsurface injection at nominal concentrations of 0.3-300 mg/kg, and the effects of creosote on aquatic plants, invertebrates and fish were assessed by sampling during a 6-wk post-treatment phase. Parameters measured included: survival of caged fish (fathead minnows and goldfish), size-age class of juvenile fathead minnows, diversity and abundance of invertebrates (zooplankton and benthic invertebrates) and phytoplankton, and biomass of macrophytes. Work subsequent to the 1994 field component has focused on the selection of bioindicators based on known effects of PAHs on aquatic organisms and on examination of data generated in the first field season. These bioindicators include: oxidative stress and sex steroid hormones in fish; membrane permeability in plants, invertebrates and fish; stress proteins in invertebrates; and fluorescence induction in algae and macrophytes. Testing the ability of the selected bioindicators to provide early warning of toxicity to populations and communities and to predict effects at higher levels of organization is the ultimate goal of this project.

QUANTIFICATION OF 15 POLYCYCLIC AROMATIC HYDROCARBONS IN CREOSOTE-CONTAMINATED WATER AND SEDIMENTS OVER TIME BY HPLC-FLUORESCENCE DETECTOR.

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Experimental design, sampling, sample preservation, extraction with a minimum cleanup procedure and subsequent quantification of 15 priority PAHs as a result of an introduction of creosote to mesocosms are presented. Creosote introduction to the mesocosms reflects two major types of creosote contamination in the aquatic environment: [1] leaching from creosote-impregnated pilings, and [2] introduction of pure creosote to aquatic environments in a spill event, and subsequent contamination of sediments by creosote. Doses for the creosote-impregnated pilings study consisted of seven levels with mesocosms containing 1 to 8 piling each, and doses for the liquid creosote study consisted of a logarithmic nominal concentration gradient from 0.3-300 mg/kg creosote. The creosote-impregnated pilings study revealed that the concentration of PAHs in the water column increased steadily with time, reaching maximum levels 7 d post-treatment, and declining thereafter. The liquid creosote study revealed that the high initial post-treatment concentration of PAHs decreased exponentially with time. The sediment data from the creosote-impregnated pilings study showed that the total amount of 15 PAHs ranged from 0.6-2 weight, while the sediment data from the liquid creosote study showed that the total amount of 15 PAHs were much higher than the pilings study, i.e. ranging from 0.6-400 weight at 4 wk post-treatment. PAHs with higher molecular weight (5 and 6 aromatic ring structures) were present at very low to nondetectable concentrations in the water column; and significantly higher levels in the sediments.

CRANKCASE OIL, HYDROCARBONS, THE ENVIRONMENT AND RAINBOW TROUT.

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Crankcase oils are lubricating oils used to minimize friction within a variety of terrestrial, marine and aeronautic vehicles (Shubkin 1993). These oils generally contain two groups of components, hydrocarbons and additives, which provide specific characteristics, such as anti-rust, anti-fungal or anti-foam properties and contribute to enhance the performance of engines (Fig. 1). Soluble additives and polycyclic aromatic compounds (PAC) can become bioavailable to marine biota and are of toxicological interest.

It has been reported that over 25-50% of the produced crankcase oil can end up in the environment, as a result of leakage, spill or from engine exhaust (EPA 1992). The short-term induction of the mixed-function oxygenase (MFO) enzyme, 7-ethoxyresorufin *-O-* deethylase (EROD) and the production of bile metabolites in trout exposed intragastricaly to used crankcase oil was previously investigated in our laboratory (Upshall et al. 1993). The presence of hydrocarbon biomarkers, the triterpanes, deriving from crankcase oil has also been examined in several sediments collected around the island of Newfoundland (Bieger et al. 1995). In a different study of the source of PAH in our local environment, it was determined that between 20-50% of 4 and 5-ring PAH detected in St. John's harbour sediments originate from crankcase oil (O'Malley et al. 1995). These studies point to the importance of investigating the fate of crankcase oil in the environment.

The present study determined the long-term biological, chemical and biochemical response of trout exposed to various concentrations of crankcase oil through the diet (referred to as E-3, E-9, E-18 and E-27 in Fig.2). This approach was chosen because exposure would be expected to occur more readily if the food items ingested by trout are coated with oil, rather than by uptake of the water soluble fraction through the gills. Trout were maintained at 10°C and sampling took place after 3, 7, 11 and 15 wks. Analyses included the determination of growth (somatic indices) and of enzymatic activity in either liver or muscle (EROD and AChE, Fig. 2). The presence of aromatic compounds was investigated in muscle and gall bladder bile (total and specific aromatic compounds) and the concentration of a series of elements in muscle (Table 1 and Fig. 3).

Table 1. Uptake of aromatics from crankcase oil by trout after 1 to 4 months exposure.

	Mean exposure (ug oil/g fish/day)			
	Control	0.0036	0.0086	0.013
Muscle conc., ug CH units/g dry wt	0.28-0.34	0.24-0.50	0.29-0.56	0.48-1.1

Table 2. Uptake of aromatics from crankcase oil by crabs after exposure to 0.025ug oil/g soft tissue/week for 100 days, followed by 0.013 ug oil/g soft tissue /week for an additional 105 days.

	Days							
	5	8	12	26	40	68	178	206
Muscle	ND	ND	ND	ND	ND	ND	ND	ND
Hepatopancreas, ug chrysene units/g dry wt	1	1	2.5	3.0	1.5	7.5	6.5	11

Snow crabs were also fed capelin containing crankcase oil, at 0-2°C, for 6 months. Feeding took place once a week, for three months and every other week, for three more months. Muscle and hepatopancreas were sampled on 8 occasions (Table 2). There was a statistically significant increase in the level of aromatics present in muscle of E-18 and E-27 trout, with time and a significant increase wih dose, at each time (weighted least square regression). The threshold level of uptake in trout was very similar to the exposure dose of crabs where accumulation was only observed in the hepatopancreas. The respiration rate of trout and crab was used to extrapolate the dietary concentration of oil into a concentration in the aquatic environment. When concentrations are expressed in terms of the various components of crankcase oil and compared to the level of aromatics reported for water runoff, it represents a 250 to 1000 times level of dilution (Hunter et al. 1979).

Spearman rank correlations were conducted between the various parameters as well as one way analyses of variance followed by studentized Tuckey range test, to determine differences between means. Some observation need further investigations to allow accurate interpretation, however in most cases there was a significant effect at the highest exposure.

The increased accumulation observed in time, for both species allows us to conclude that equilibrium is not reached after 4 mo. Chemical analyses of the muscle and gall bladder bile content of trout, and hepatopancreas content of crabs raises questions as to the nature of the aromatics that bioaccumulate. Analysis for aliphatic and aromatic hydrocarbons, the major constituents of the oil proved negative and correlation between their concentration in oil, their physical-chemical properties (water solubility or octanol-water partition coefficient) supports their non-detectable levels. More research into the identity and quantity of the various chemical classes used as additives in the oil and their fate in the environment is warranted.

Acknowledgements

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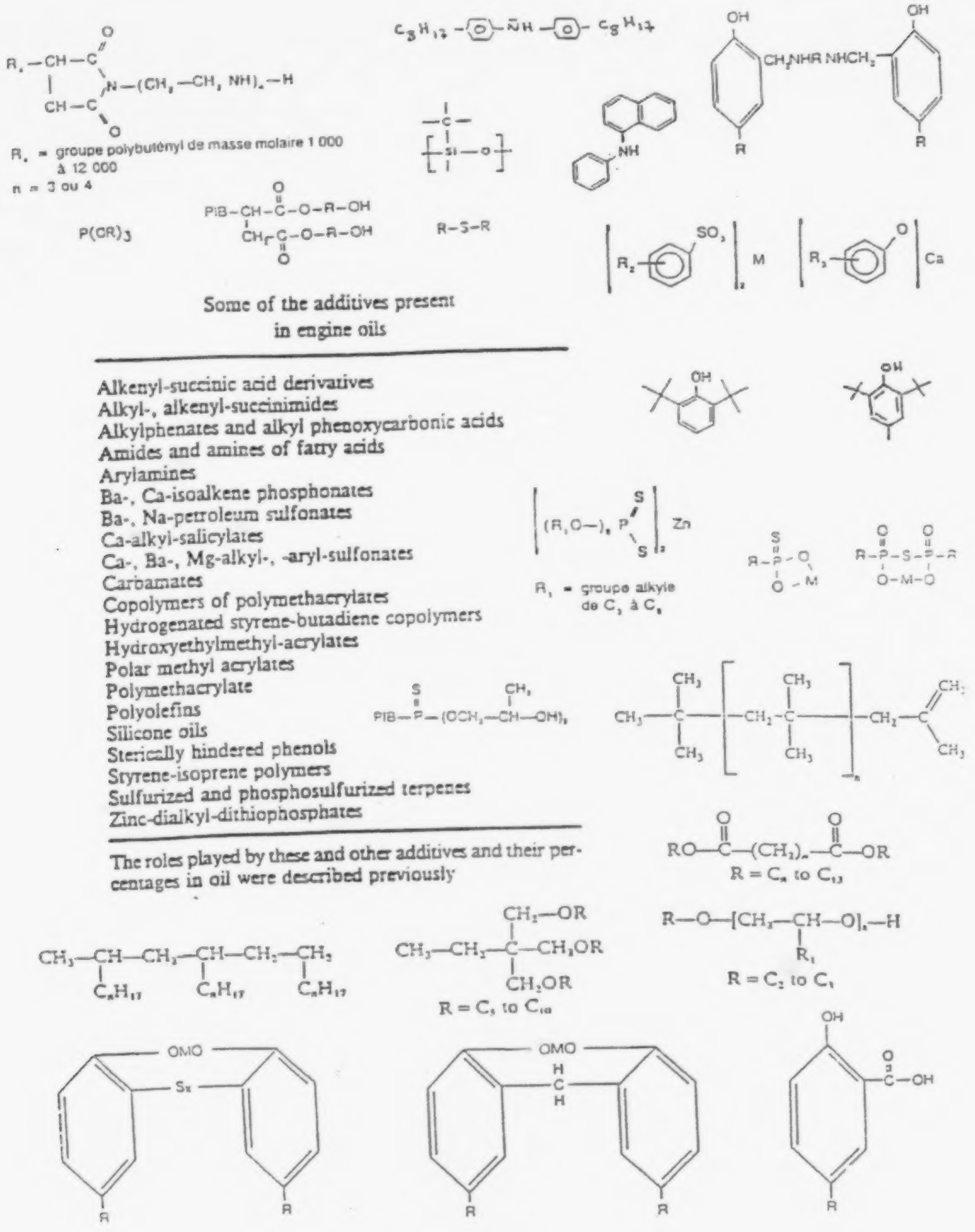
Fig. 1. Chemical classes and structure of a variety of additives present in engine oil.


R₁ = groupe polybutényl de masse molaire 1 000 à 12 000
n = 3 ou 4
P(OR)₃
R–S–R
R₁ = groupe alkyle de C₁ à C₈
R = C₄ to C₁₃
R = C₁ to C₁₀
R = C₂ to C₁

Some of the additives present in engine oils

- Alkenyl-succinic acid derivatives
- Alkyl-, alkenyl-succinimides
- Alkylphenates and alkyl phenoxycarbonic acids
- Amides and amines of fatty acids
- Arylamines
- Ba-, Ca-isoalkene phosphonates
- Ba-, Na-petroleum sulfonates
- Ca-alkyl-salicylates
- Ca-, Ba-, Mg-alkyl-, -aryl-sulfonates
- Carbamates
- Copolymers of polymethacrylates
- Hydrogenated styrene-butadiene copolymers
- Hydroxyethylmethyl-acrylates
- Polar methyl acrylates
- Polymethacrylate
- Polyolefins
- Silicone oils
- Sterically hindered phenols
- Styrene-isoprene polymers
- Sulfurized and phosphosulfurized terpenes
- Zinc-dialkyl-dithiophosphates

The roles played by these and other additives and their percentages in oil were described previously

Fig. 2. Box and whisker plots presenting experimental data. Numbers following E and H refer to the level of exposure (3, 9, 18 and 27). Letters following the numbers reflect the sampling time (A,B,C and D).

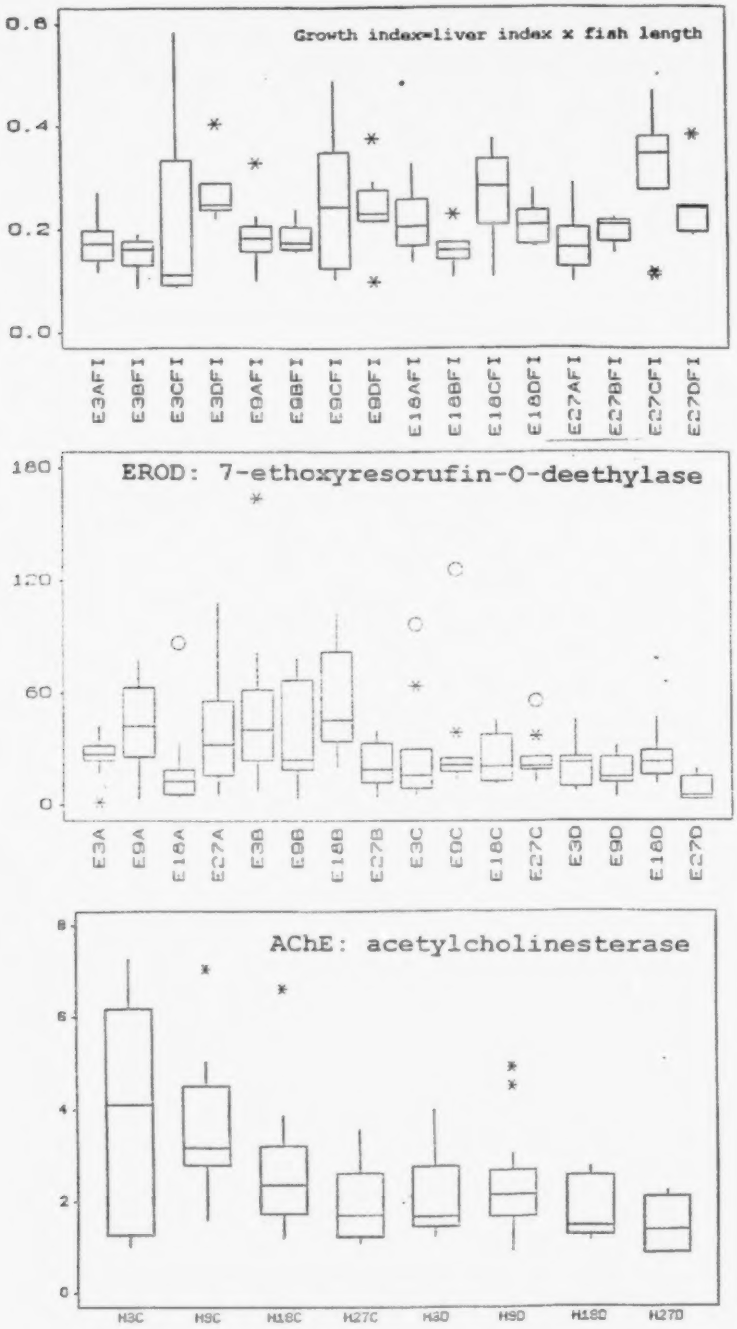
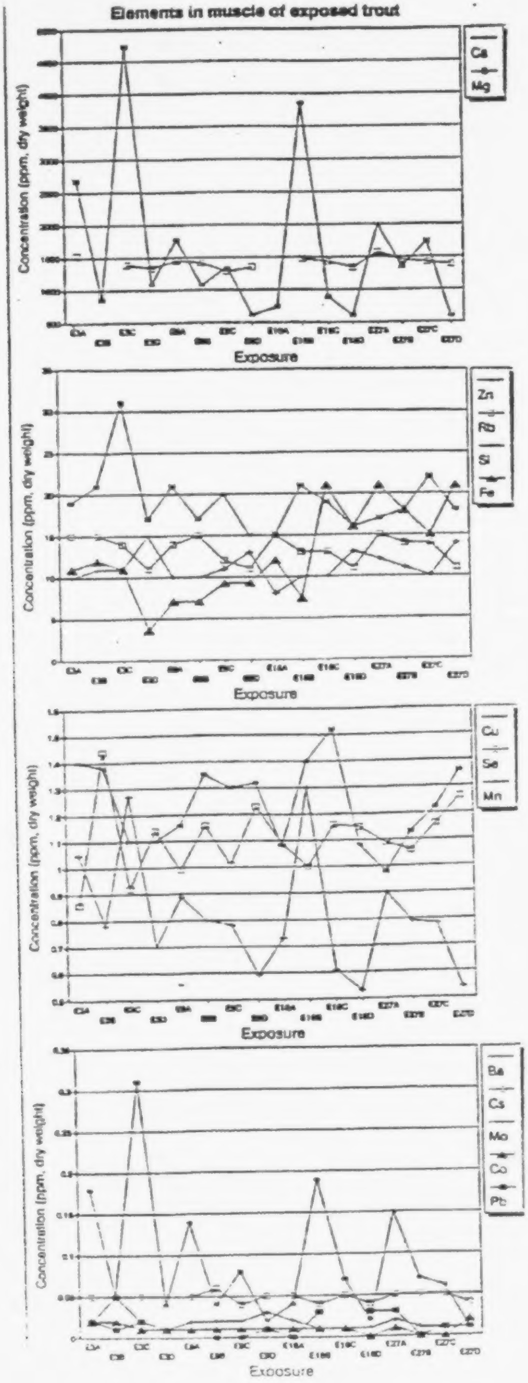


Fig. 3. Concentration (mg/kg, dry weight) of elements in pooled muscle samples (by exposure level and by sampling time) obtained from rainbow trout.



PAH BILE-METABOLITES IN WINTER FLOUNDER (*Pleuronectes americanus*) FROM A COAL-TAR CONTAMINATED ESTUARY AND AFTER EXPERIMENTAL EXPOSURE TO CONTAMINATED SEDIMENTS IN THE LABORATORY.

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Sediments in Sydney estuary (Nova Scotia) have been chronically contaminated with coal-tar residues since 1890 from a point-source tar pond in Muggah Creek. In other studies we have demonstrated a pronounced MFO gradient in flounder taken from a sediment-PAH gradient within the estuary. The presence of bile-metabolites are a direct indication of metabolic response to PAH. In this study we measured 12 PAH metabolites in bile from fish from Sydney estuary, from reference stock and from fish deliberately exposed for 3 wk to coal-tar contaminated sediments. Background PAH metabolite concentrations in laboratory held fish (on clean sediment) averaged from 0.55 mg/kg to below detection levels.

Experimental fish, exposed for 3 wk on point source sediments taken from Muggah Creek, showed bile levels of 1- and 2-naphthol (1 mg/kg 4-phenylphenol (1-5 mg/kg), anthraquinone (<2 mg/kg), OH-fluoranthene (2-7 mg/kg), anthraldehyde isomers (5 mg/kg), OH-pyrene (12 mg/kg) and phenanthrol (20-60 mg/kg). By contrast, flounder taken directly from the coal-tar gradient in the estuary showed low (0.64 mg/kg) to negligible metabolites in bile, with values similar to those determined in reference fish. We believe that the high bile-metabolites levels in the experimental exposed fish represent the worst case scenario of flounder living in and near the tar pond. In fact, in the field we were unable to find flounder anywhere near the tar pond point source. The lower levels of bile-metabolites in fish taken from elsewhere in the estuary may reflect a life habit where fish actively avoid highly contaminated sediment, even in stations with demonstrate high PAH-sediment concentrations. There is also the possibility that previously exposed and induced fish may metabolize PAH more readily.

INDUCTION OF FISH HEPATIC MIXED FUNCTION OXYGENASE (MFO) BY SUBSTITUTED POLYAROMATIC HYDROCARBONS (PAHs).

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Rainbow trout (*Oncorhynchus mykiss*) were exposed in the laboratory to pure compounds at nominal concentrations of 0.1-1,000 μg/L and hepatic MFO activity was measured after 4 d. Substituted PAHs that induced ethoxyresorufin-*O*-deethylase (EROD) were 2,3,6-trimethyl naphthalene, 3,6-dimethyl phenanthrene and 1-methyl-7-isopropyl phenanthrene (retene). No EROD induction was detected in fish exposed to naphthalene or phenanthrene. In a study examining effluents from four oil refineries in Ontario, laboratory fish exposed for 4 d to 1-100% effluent also showed dose-related increases in EROD activity, with significant induction above control levels usually seen at about 32% effluent. Investigations into the types of compounds present in the refinery effluents are ongoing. None of the EPA priority PAHs were detected in any of the refinery effluents at concentrations above low ppt for each individual PAH. In another study, wastewater from an oil sands mining and upgrading facility was examined for ability to induce MFO in fish. Semipermeable membrane devices (SPMDs) were used to concentrate compounds in the effluent for 14 d. Extracts of exposed SPMDs induced EROD in a fish cell line (*Poeciliopsis lucida* hepatic carcinoma, PLHC-1) and were 23 x more potent than extracts of SPMDs exposed to river waters in the oil sands area. Concentrations of parent PAHs in the SPMD extracts were low compared to concentrations of C1 to C4 substituted PAHs. From these studies it appears that some substituted PAHs may induce MFO activity in fish. Little is known about the fate of these compounds in the environment or about their potential for causing biological effects.

EVALUATION OF FISH POPULATION EFFECTS DUE TO CREOSOTE EXPOSURE IN AQUATIC MESOCOSMS.

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Studies involving controlled exposures to polyaromatic hydrocarbons have typically studied the effects of exposure to individual compounds. However, PAHs are usually present in the environment in complex mixtures. Some of these (e.g. creosote) have been widely used and present potential risks to aquatic ecosystems. The use of replicate mesocosms (precolonized with zooplankton, phytoplankton, macroinvertebrates, and periphyton) enabled evaluation of the total population impact resulting from creosote exposure in two species of fish, integrating both direct toxic effects and indirect community-level interactions. Two methods of creosote addition were used, resulting in two series of mesocosm exposures: nine ponds were dosed with liquid creosote (from 0-300 mg/kg per pond), and nine were dosed using creosote-impregnated marine pilings (0-8 pilings per pond). Mortality of two species of fish, *Carassius auratus* and *Pimephales promelas*, was monitored over time and subsequently evaluated in terms of time profiles for zooplankton density and aqueous concentrations of the 15 most abundant PAHs present in creosote. In addition, the chronic effect to the juvenile fish population was studied.

EFFECTS OF SEDIMENT PAH CONTAMINATION ON THE VITELLOGENESIS IN WINTER FLOUNDER (*Pleuronectes americanus*).

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The sediments of Sydney Harbour (Nova Scotia) are among the most contaminated with polycyclic aromatic hydrocarbons (PAH) in Canada. Levels of up to 500 mg/kg have been reported at the point source of the contamination (coal-tar settling pond). Some of the reproductive parameters of female winter flounder (*Pleuronectes americanus*) from the Sydney Estuary were studied seasonally for two consecutive years. The fish were captured by trawl from four stations in the harbour and one station in St. George's Bay (control site).

The results suggest the existence of a threshold level of contaminant above which the reproductive cycle of the female flounder (vitellogenesis) is affected. For fish originating from sampling stations with a PAH sediment concentration at or above 10 mg/kg we were able to detect a significant reduction of estradiol concentration in the serum and a significant retardation of egg maturation. Several individuals from the most contaminated sites also displayed inhibition of spawning. However, no accumulation of PAH in the gonadal tissue was detected, and the levels of PAH metabolites in the bile were low. This would suggest that the disruption linked to PAH contamination occurs during one of the initial phases of the reproductive cycle in spite of active metabolization and excretion of the contaminants to which the fish are exposed. This could mean that there exists a potential for recovery or compensation.

THE USE OF MEMBRANE INTEGRITY AS A BIOINDICATOR OF CREOSOTE EXPOSURE AND EFFECTS.

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Abstract

The University of Guelph's Centre for Toxicology is conducting a study to develop and validate bioindicators of creosote exposure and effects in aquatic systems using mesocosms. Creosote is a mixture of polycyclic aromatic hydrocarbons (PAHs) and other compounds. Previous studies have indicated that some PAHs interact with biological membranes, causing changes in membrane integrity. Changes in membrane integrity were determined by K^+ leakage measured by atomic adsorption spectrophotometry and fluorescence polarization spectrofluorometry in both *Myriophyllum* sp. and *Hexagenia* sp. Results from the 1995 field season indicate some changes in membrane integrity in *Myriophyllum* sp. No membrane effects were observed in *Hexagenia* sp.

Introduction

The University of Guelph Centre for Toxicology is conducting a study to develop and validate bioindicators of creosote exposure and effects. The focus is on bioindicators that are measurable at lower levels of ecosystem organization (i.e. organismal), at lower doses, or in a shorter time frame, but are predictive of effects at higher levels of organisation (i.e. population or community). The study is being conducted at the aquatic mesocosm facility at the University of Guelph, Guelph, Ontario.

Creosote, which consists of over 300 individual compounds, is composed of approximately 85% polycyclic aromatic hydrocarbons (PAHs), 10% phenolics and 5% heterocyclics (Environment Canada 1994). PAHs can disrupt biological membranes (Van Overbeek and Blondeau 1954), increasing their permeability and fluidity (Sikkema et al. 1994). As well, acridine, a component of creosote, has been shown to increase the permeability of the plasma membrane in *E. coli* (Wagner et al. 1980). It was hypothesized that membrane integrity, as measured by membrane potassium ion permeability and membrane fluidity, may be used as a bioindicator of the effects of creosote in freshwater aquatic systems.

Materials and Methods

Organisms

A rooted aquatic macrophyte,*Myriophyllum* sp., and a benthic invertebrate, the mayfly nymph *Hexagenia* sp. were used in this study. Four stalks of *Myriophyllum* in pots were added to each mesocosm four weeks prior to dosing. *Hexagenia* were laboratory reared from eggs and obtained from the Canada Centre for Inland Waters in Burlington, Ontario, and introduced to the mesocosms one wk prior to dosing. *Hexagenia* were caged in plastic containers and fitted with a screen lid containing 5 cm of sediment. Fifty nymphs were placed in each cage, giving a final density of greater than $9cm^2$ sediment per nymph.

Sampling

A description of the mesocosms and experimental design can be found in Robinson et al. (1994) with the exception that 16 mesocosms were used for the liquid creosote study in 1995 with a dose range of 0.053 ml/L to 100 ml/L plus two controls. Samples were collected 2, 4 and 8 weeks following application of creosote to the mesocosms.

The concentrations for the *Myriophyllum* ranged from 0.053 ml/L to 100 ml/L plus two controls. For each sampling period, approximately 20 cm from one to two healthy-looking plants was sampled. Approximately 125 mg of tissue from the tip of the plant was used for the K^+ leakage assay. The remainder of the tissue was used for the fluidity assay. The concentrations for *Hexagenia* ranged from 0.053 ml/L to 56 ml/L plus one control. One *Hexagenia* cage was sampled for each assay.

K^+ Leakage Assay

A *Myriophyllum* tissue sample or 15-30 *Hexagenia* nymphs were placed in 40 mL of K^+ free media (double distilled water for *Myriophyllum*; artificial pond water (Sprung 1987) made K^+-free by omitting KCO_3 for *Hexagenia*) and allowed to stand for 24 h, after which a 20 mL subsample was taken. Twenty mL of media was added such that the total volume remained at 40 mL. The samples were boiled (20 min for *Myriophyllum* ; 10 min for *Hexagenia*) and allowed to stand another 24 h, after which a 20 mL subsample was taken. All subsamples were acidified with 100 μL of fuming nitric acid (Fisher Reagent A.C.S.) and refrigerated until analyzed by atomic adsorption using an air/acetylene flame (Perkin-Elmer 2380 Atomic Adsorption Spectrophotometer, Hamamatsu Photonics K.K. K^+ lamp). All samples were analyzed within 5 d of being taken. The percent of K^+ leaked by the membranes was calculated by taking a ratio of the amount of potassium leaked by the membrane prior to boiling to the total amount leaked after boiling.

Fluidity Assay

Myriophyllum stems or whole *Hexagenia* nymphs were homogenized in a 30M sucrose in 50 mM MOPS (Sigma) buffer at pH 7.0 and then filtered through four layers of cheesecloth. The solution was then centrifuged (12000 rpm in a microcentrifuge for 10 min for *Myriophyllum*; 10000 g for 20 min for *Hexagenia*) and the supernatant taken. Protein in the supernatant was determined according to Bradford (1976) and a concentration of 100 μg protein/mL was used in the assay. The steady-state fluorescence polarization was then measured as described by Duxbury (1988) with the modification that 50 mM MOPS was used as the buffer. All sample preparation was done on ice. The degree of polarization was calculated form Shinitzky and Barenholz (1978).

Results

Myriophyllum sp.

A dose-response decrease in the biomass of *Myriophyllum* was seen at the end of the 1994 field season, indicating that creosote does affect growth of this species. However, no change in membrane fluidity was observed 2 and 4 weeks following creosote addition to the mesocosms during the 1995 field season. An increase in fluidity (as shown by a decrease in microviscosity) was observed at the highest concentrations eight weeks after creosote addition (Fig. 1). A trend towards increased potassium leakage was also observed at both two and eight weeks after dosing, but not at four weeks (Fig. 2).

Hexagenia sp.

A dose-response decrease in *Hexagenia* survival in the cages was observed during the 1995 field season, with the creosote affecting survival in the range of 0.5-5.5 ml/L. However, unlike *Myriophyllum*, no dose-response changes in either membrane fluidity or membrane potassium leakage were observed (Figs. 3 and 4).

Discussion

Changes in *Myriophyllum* membrane fluidity and potassium leakage were observed at the higher doses of creosote in the mesocosms (>3 ml/L in the mesocosm). Due to the loss of some samples at the higher concentrations, these changes may not have always been detected. This may have been the case for the potassium leakage assay four weeks after creosote addition. As well, due to the gross effects of the creosote in the mesocosms on the plants immediately following dosing (i.e. plant death at the higher concentrations), there was often not enough live tissue left to conduct a fluidity assay. Thus, changes in fluidity at these higher doses would not be measured.

No observable effects on membranes were observed with the *Hexagenia* despite changes in survival. However, only one of the doses used actually fell within the survival response curve. All other doses used either had 100% survival or 100% mortality. Thus, any changes may not have been detected with the doses used.

In conclusion, effects were observed for both test organisms at the population level. There appeared to be an increase in both membrane fluidity and potassium leakage in *Myriophyllum* at the higher concentrations. No changes were observed in *Hexagenia*. Further studies will be conducted in the laboratory to better define the dose-response curve.

Acknowledgements

This project was supported by an NSERC graduate student grant and by the Canadian Network of Toxicology Centres. I would also like to thank Ketut Bestari, Bob Gensemer, James Jupp, Kim Munro, Kelly Postma, Al Shaw, Tracey Steele and Peter Takacs for their help and support.

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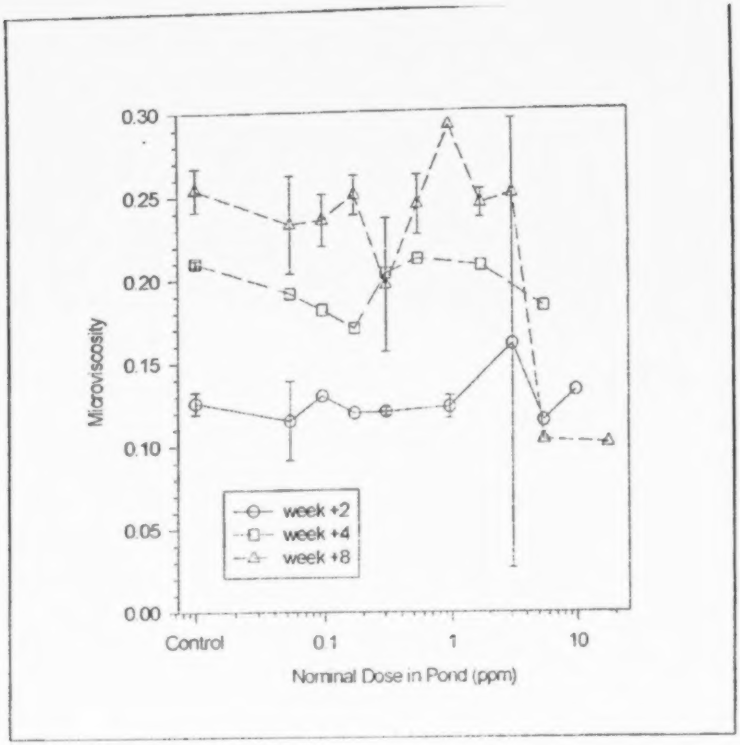

Figure 1: Changes in *Myriophyllum* sp. membrane microviscosity with time after applicati of creosote to aquatic mesocosms during the 1995 field season

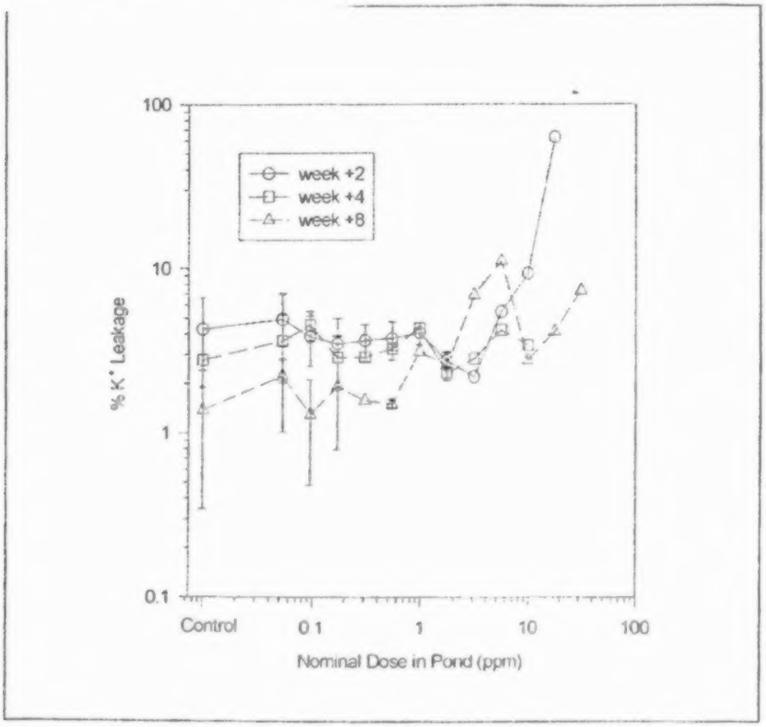

Figure 2: Changes in *Myriophyllum* sp. membrane potassium leakage with time after application of creosote to aquatic mesocosms during the 1995 field season.

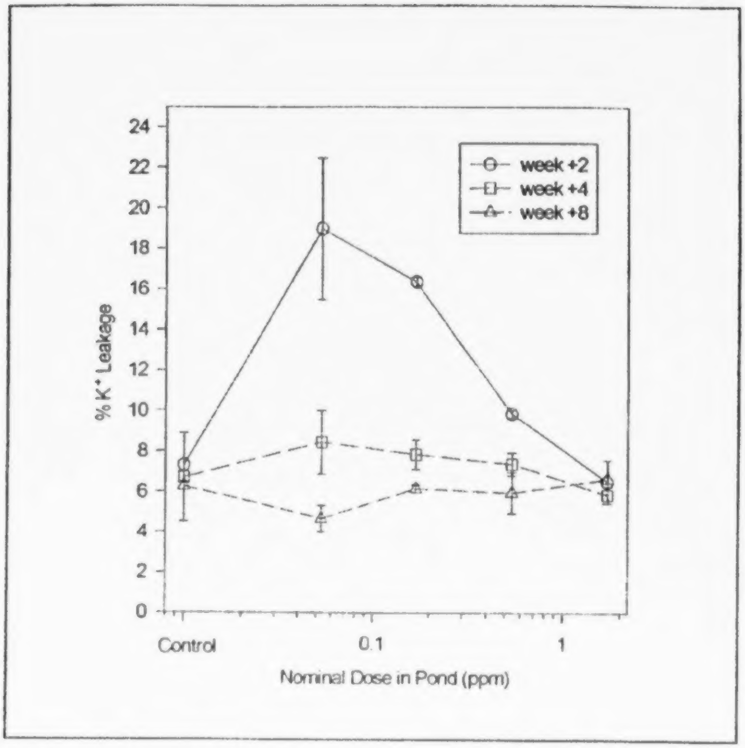

Figure 3: Changes in Hexagenia sp. membrane microviscosity with time after application of creosote to aquatic mesoscosms during the 1995 field season.

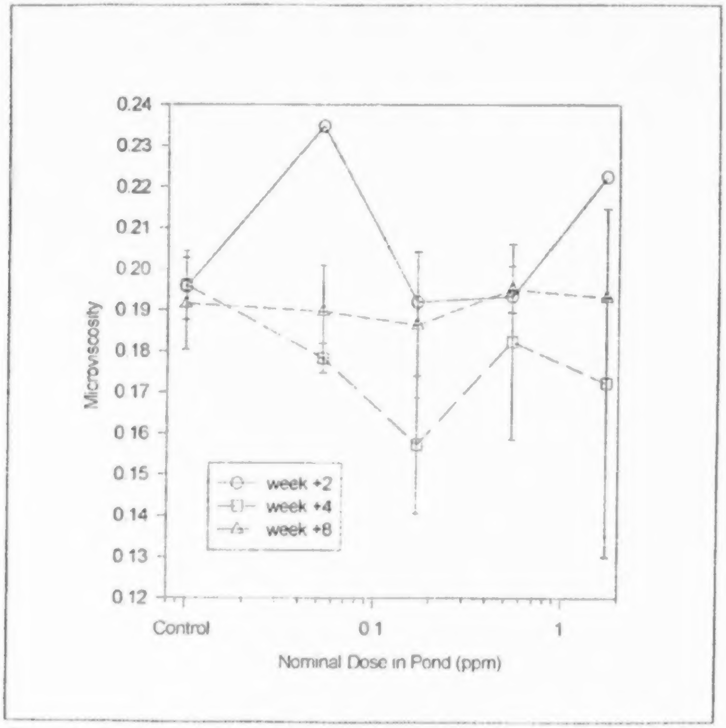

Figure 4: Changes in Hexagenia sp. membrane potassium leakage with time after application of creosote to aquatic mesocosms during the 1995 field season.

FLUORESCENCE INDUCTION AS A BIOINDICATOR OF CREOSOTE TOXICITY IN PLANTS.

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The use of chlorophyll fluorescence induction as a photosynthetic bioindicator was evaluated as part of a study to assess the effects of polycyclic aromatic hydrocarbons (PAHs) on aquatic macrophytes. The wood preservative creosote was used as a mixed PAH source. Toxicity to aquatic plants was measured at the biomarker level using the chlorophyll fluorescence induction assay and compared to effects at the population level in outdoor mesocosms. The aquatic macrophytes *Lemna gibba* and *Myriophyllum* sp. were exposed to liquid creosote at nominal concentrations of 0.1-100 L/L in static renewal 8 d toxicity bioassays. Plants were incubated in simulated solar radiation, which mimics the relative levels of UV in natural sunlight. Population-level endpoints (growth rate, chlorophyll content) were compared to results from the fluorescence induction assay. Growth of plants was inhibited by creosote at concentrations above 3 L/L. However, inhibition of photosynthesis was detected by fluorescence induction at much lower creosote concentrations. Fluorescence induction assays of plants exposed to creosote in outdoor mesocosms were predictive of population-level effects, and detected photosynthetic inhibition at lower creosote concentrations than growth assays. In addition, damage to the photosynthetic apparatus was detected after only a few hours of exposure to creosote. Chlorophyll fluorescence therefore appears to be a rapid and sensitive bioindicator for the toxicity of a PAH mixture, and is consistent with results from growth-based macrophyte bioassays.

PCBS IN WATERWAYS: TRANSPORT AND TOXICITY
SESSION CHAIR: B. BUSH

PCB CONTAMINATION OF THE ST. LAWRENCE RIVERWAY AND THE MOHAWK ECOSYSTEM.

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Polychlorinated biphenyls (PCBs) are widespread, persistent environmental contaminants and their toxicity to wildlife and humans is of major concern. The PCB contamination of the Mohawk Nations Lands and Waterways has ended a people's tradition of subsistence fishing and land usage.

For the past 4 yr, we have conducted a series of environmental sampling around the GM landfill and cove site known as "Contaminant Cove" adjacent to the Akwesasne Mohawk Nation. Form this site and the surrounding area, we have looked at PCB transport, totals and patterns. The sampling included air, water, sediment, weeds, fish, ducks, frogs, caddisflies, ants, earthworms, soil, grass, maggots, sow bugs and locally grown vegetables. The samples were analyzed on a Hewlett Packard 5890 GC/ECD using a DB-5 capillary column. The analyses were calibrated using a 1:1:1:1 mixture of Arochlors 1221, 1016, 1254 and 1260 (200 ng/mL each), spiked with 10 ng/mL p,p'DDE, Mirex and 5 ng/mL hexachlorobenzene (HCB). Seventy-one PCB peaks, representing 68 congeners, DDE, Mirex and HCB, were determined for all the samples.

By comparing the patterns of the individual PCB congeners from sample to sample, it is shown that the total PCB concentration may differ but the pattern remains the same. The use of a congener-specific method allows us to see the new patterns of the original Aroclor used and the lipophilic nature of each PCB congener in the ecosystem. The detailed PCB congeners profile of the riverway and its ecosystem reflects the bioconcentration and bioaccumulation in that environment.

PATTERN ANALYSIS OF PCBs IN LAKES, RIVERS, OCEANS, AND TERRESTRIAL SYSTEMS

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A pattern analysis was done on data, published and unpublished, concerning PCB patterns in insects, snails, mammals and other components of the various systems. Analysis was by pattern analysis derived from community similarity analyses (as derived from ecology) and presumed a lack of linear relationships. Cluster analysis was done on some of the data to show relationships in the multidimensional data sets. The results point to a separation of individual contaminants after release to the ecosystem between organisms, and even organs within organisms, as well as components of the systems. Higher K_{ow} or higher chlorine content congeners tended to bioaccumulate in higher trophic levels and may converge in patterns at lofty trophic levels. Relationships between PCB contaminants and either the environment or within organisms has both a trophic hierarchy and, perhaps, organ hierarchy components to the redistributions. Use of indicator organisms or organs of organisms (muscle) should be approached with extreme caution.

CURRENT PCB DEPOSITION IN SNOW IN THE YUKON TERRITORY.

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[3]Environment Canada, Edmonton, AB.

In the Yukon and in much of Canada, snowfall is an important component of the total annual precipitation. Consequently, snowmelt during the spring is the single most important hydrological event throughout most of Canada, including the Yukon Territory. As part of the Arctic Environmental Strategy, a study was undertaken to quantify the annual deposition of trace organic contaminants. Objectives of the study were to quantify annual deposition of contaminants with the establishment of a multi-station snowpack network and to quantify the weekly deposition of contaminants during the winter by operating three snow collectors at selected intensive sampling locations strategically located within the Territory. Snow samples collected were analyzed for 67 of the possible 209 PCB congeners. The PCB congener patterns for the three snow collectors were comparable, furthermore, individual sample PCB homolog concentrations were generally lower in the bulk samples relative to the snow collectors. Each site demonstrated a general increase in concentration for the homologs with up to four chlorine atoms (i.e. mono through tetra). The bulk samples were similar in homolog concentrations; however, differences existed between the White Pass site and Dawson City site; White Pass was shown to have higher concentrations of 1 and 2 chlorinated congeners. Concentrations of PCBs were an order of magnitude lower in bulk samples relative to the White Pass site. Mean sum PCB deposition was 4.4 and 3.6 ng/m^2/d at Tagish and Whitehorse, respectively. These values are quite comparable with High Arctic sites from the NWT. In contrast, the deposition at Whitepass was estimated at 100 ng/m^2/d. The deposition at Tagish and Whitehorse agreed well with the snowpack samples at Tagish, Beaver Creek and Burwash Creek, ranging between 2.3-5.4 ng/m^2/d. Interpretations of differences in deposition of PCBs will be discussed.

SORPTION AND DESORPTION OF PCB CONGENERS FROM WATER BY CHLORITE, ILLITE AND MONTORILLONITE CLAY.

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An aqueous solution of a mixture of Aroclor 1242 and 1254 (4:1 by weight) with sediment concentrations ranging from 0.26-10.0 g/L of a 5-μ natural clay sample of low organic carbon content were employed. Major constituents of the clay obtained from Coeyman's, New York, were chlorite, illite and montmorillonite. A sorption isotherm was determined to yield K_d and l/n values for the Freundlich equation at a sediment concentration of 2.0 g/L and PCB from 1.5-10.0 ng/mL. Sorption was studied at constant PCB concentration between 1.5 and 3.0 ng/mL with sediment ranging from 0.26-10.0 g/L for periods of 1, 7 and 30 da. A decrease in K_d was noted with increasing sediment concentration. This became less pronounced with increasing contact time, when the K_d at all sediment concentrations approach one value. A successive batch desorption experiment was conducted over a range of 0.26-10.0 g/L for 2, 10, 20 and 30 d. After the 30 d, the clay was analyzed to determine the concentration of strongly sorbed PCBs. With increasing sediment concentration, the weakly sorbed PCB component decreased, whereas the strongly sorbed PCB component increased. The relevance of these results to PCB transport in the Hudson River will be discussed.

PCB CONTAMINATION OF SEALS INHABITING CENTRAL AND NORTH PACIFIC COASTAL WATERS.

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Marine mammals are known to bioaccumulate PCBs and other lipophilic contaminants through the food chain and pass them on to their young in appreciable quantities, both in utero and via mother's milk. The toxic effects of perinatal PCB contamination in marine animals are not well understood. In this study, PCB congeners and coplanar PCBs were analyzed in the blubber of perinatally exposed seals from two geographical areas of the US Pacific coast. Group A comprised eight harbor seal (*Phoca vitulina*) pups that were sampled shortly after death on two islands in the Puget Sound (WA) estuary. Group B comprised 10 northern elephant seal (*Mirounga angustirostris*) and 11 harbor seal pups that were sampled both alive and at necropsy at a rehabilitation facility north of San Francisco (CA). For Group B, various biomarkers of effect (endocrine, immune, hematological and clinical chemistry markers) were also measured. TCDD toxic equivalency (TEQ) values were calculated to compare the relative potency of coplanar PCB residues detected in blubber. Significant geographical and species variations were observed for both groups. The most potent congeners in all harbor seal specimens were 345,34-pentachlorobiphenyl and 2345,34-hexachlorobiphenyl (IUPAC nos. 126 and 156), whereas in elephant seals, 245,34-pentachlorobiphenyl (IUPAC no. 118) contributed the greatest proportion of the TEQ, followed by IUPAC no. 126. These data show that seals are an important indicator species for dioxin-like PCBs in marine and estuarine waters. Preliminary analyses have also revealed statistically relevant relationships between coplanar PCBs and thyroid and immune function markers in California seals.

MERCURY IN AQUATIC ECOSYSTEMS
SESSION CHAIR: W. PILGRIM

ECOSYSTEM ASPECTS OF MERCURY POLLUTION

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Abstract

In the past, mercury pollution was a serious problem near point sources that discharged inorganic mercury to the aqueous environment. There is now a renewed concern that mercury pollution from atmospheric sources may have created wide scale regional and global pollution. Scientists have shown from the analyses of lake sediment cores that mercury deposition has increased from the beginning of the industrial period. Biological effects caused by the observed increase in mercury deposition are not well defined. To help focus the mercury issue in Canada, the Ecological Monitoring Coordinating Office (EMCO) hosted a national mercury workshop in September 1995. The three primary issues addressed at the workshop were anthropogenic verses natural contributions of mercury, critical loads and regional effects and risk assessment. This paper is a short review of current information relating to this workshop and the mercury issue.

Introduction

Scientists have shown from the analyses of lake sediment cores that mercury deposition has tripled in some regions since the beginning of the industrial period, approximately 100 years ago (Figure 1); (Swain et al. 1992; Lockhart 1995; Lucotte et al. 1995). Protocols to control heavy metal emissions are being developed by many European countries. If the protocols are ratified, emission controls would also be likely for North America. However, scientists have not clearly demonstrated to policy makers the extent of the mercury problem, and the role that anthropogenic sources play, and whether or not the problem is serious and widespread enough to ask for controls on mercury emissions. Canada has no national program to assess the mercury issue, and the situation may not be the same as it is in Europe.

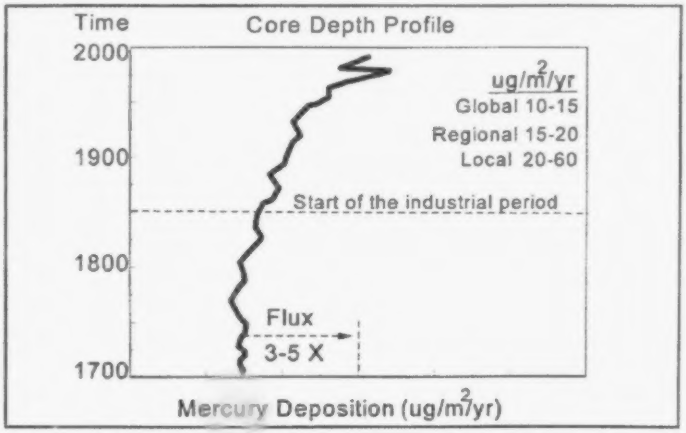


Figure 1. Historic trends in mercury deposition.

A number of knowledge gaps on mercury still exist; the association between observable effects and noted increased deposition are poorly substantiated; elemental mercury is very volatile and biogeochemical cycles between oceans, atmosphere, and soils are unclear (EPRI 1994); (Rasmussen 1994); there are local, regional, and global issues; there are multiple environmental factors influencing methylation (Swain and Hewig 1992); there are synergistic interactions between mercury and oxidants that affect deposition rates;

there are large geological anomalies in Canada with naturally high background levels of mercury which obscure anthropogenic effects in these areas (Painter et al. 1994) and reliable techniques for accurate analysis of mercury in air and water have only been developed in the past decade.

Discussion

Sources

Recent estimates suggest that anthropogenic emissions account for 60 to 80 percent of the global mercury emissions. The total atmospheric pool is roughly estimated to be 25 megamoles (Mason et al. 1994). However, there are no exact numbers for global mercury emissions as inventories are still being completed and many small sources are often not included (Figure 2). Recent estimates suggest that Canada, the United States and Mexico emit 284 tonnes of mercury per year and including Central America and the Caribbean 336 tonnes are emitted (Porcella 1995). Porcella further estimated that this sector may only contribute 2-3 percent of elemental mercury to the global pool. The most recent estimate for Canada suggests 41 tonnes of mercury are emitted annually (Nriagu, 1994). Environment Canada's inventory being conducted by the national pollutant inventory release survey is not yet completed for mercury.

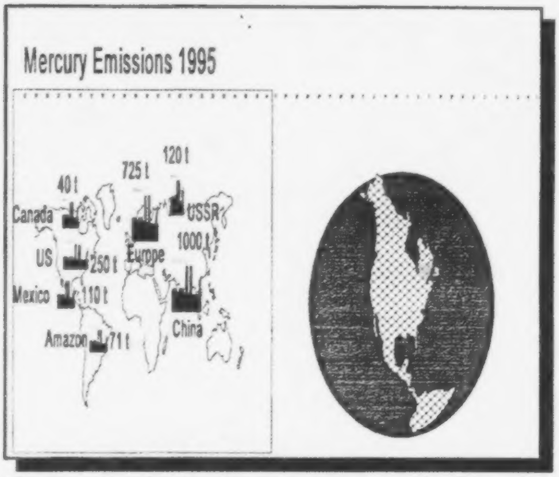
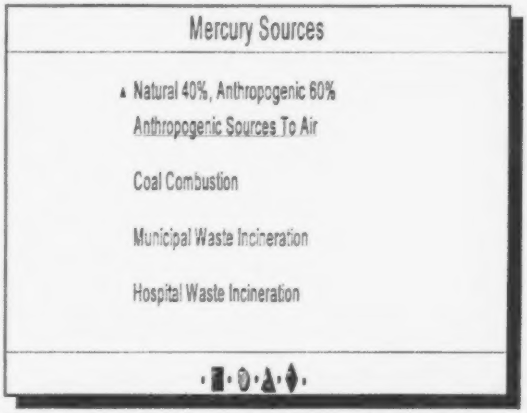


Figure 2. Estimate of some anthropogenic mercury global emissions.

Eastern North America has a combination of mercury sources, such as incineration, fuel combustion and disposal of products containing mercury (Figure 3). This northeast region has shown an increase in mercury deposition since the beginning of the industrial period (Nater and Grigal 1992).To more clearly demonstrate these trends further sediment core research is needed. Atmospheric deposition of mercury is governed by site specific emission sources, transport and transformation processes involving oxidants. In Europe, local scale deposition is suggested to occur between 0-100 kilometres of the source and the regional scale between 100 to 2000 kilometres of the source (Iverfeldt 1995). Stack height would also be important to the dispersion of particulate mercury. To accurately study spatial and temporal trends in mercury a global network is essential (Fitzgerald 1995). In the context of effects on biological systems, realistically, mercury cannot be evaluated alone as many natural systems impacted by airborne transport will have multiple organic pollutants, other heavy metals plus natural stressors.



Mercury Sources

- Natural 40%, Anthropogenic 60%

Anthropogenic Sources To Air

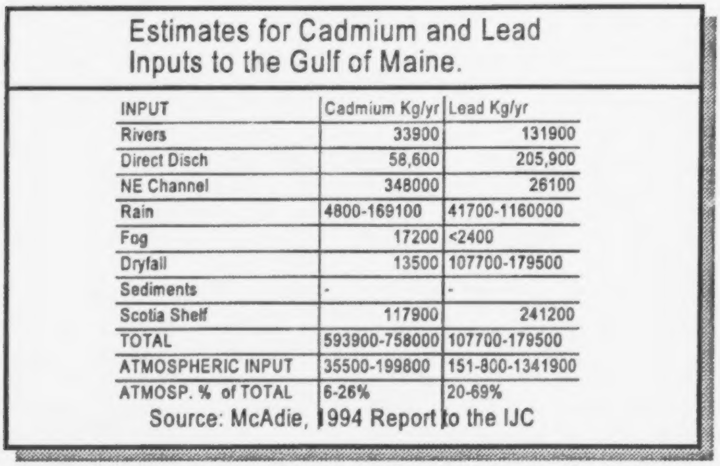
Coal Combustion

Municipal Waste Incineration

Hospital Waste Incineration

Figure 3. Primary anthropogenic sources of mercury.

Elemental mercury has a has an atmospheric residency time of about one year (Iverfeld 1995). Thus



Estimates for Cadmium and Lead Inputs to the Gulf of Maine.

INPUT	Cadmium Kg/yr	Lead Kg/yr
Rivers	33900	131900
Direct Disch	58,600	205,900
NE Channel	348000	26100
Rain	4800-169100	41700-1160000
Fog	17200	<2400
Dryfall	13500	107700-179500
Sediments	-	-
Scotia Shelf	117900	241200
TOTAL	593900-758000	107700-179500
ATMOSPHERIC INPUT	35500-199800	151-800-1341900
ATMOSP. % of TOTAL	6-26%	20-69%

Source: McAdie, 1994 Report to the IJC

Figure 4. Significance of atmospheric inputs to the Gulf of Maine (McAdie, 1994).

considering what we already know about long-range transport, atmospheric transport of mercury to Atlantic Canada from US sources is inevitable. However, spatial and temporal deposition trends for North America have not yet been established. A partial mass balance was completed on the Gulf of Maine for cadmium and lead showed that atmospheric input was an important pathway for contaminants entering the Gulf of Maine (Figure 4). However, mercury inputs were not estimated as a part of that project. In follow-up to recommendations from participants of the Gulf of Maine Conference that "the Gulf of Maine Council should work with others to improve our understanding of the magnitude of atmospheric inputs to the Gulf's watershed and marine environment", a mercury deposition network was proposed. The goal is to establish a mercury deposition network in the Gulf of Maine Airshed, with at least one coastal site in each of the GOM jurisdictions. Sites in Canada will be located at the two rural EMAN Sites; one in St. Andrews, New Brunswick and the other in Kejimkujik National Park, Nova Scotia. In the United States, sites are proposed for coastal Massachusetts and New Hampshire. These sites will supplement an existing rural NADP/MDN site operating at Acadia

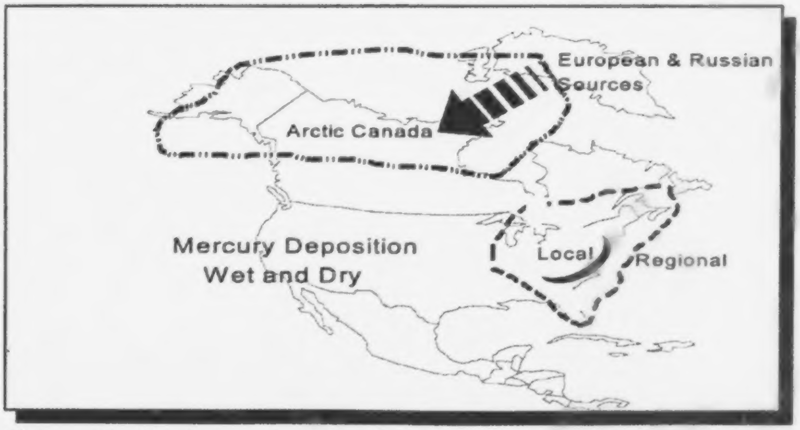


Figure 5. Regions showing increased mercury deposition in sediment cores.

National Park, Maine. The future goal is to run this project for 5 years to coincide with the NADP/Mercury Deposition Sub-network. The GOM Mercury Deposition Study is intended to be a project affiliated with the GOM Monitoring Committee and will be a joint venture between the Ecological Monitoring and Assessment Network of Canada (EMAN CANADA), Atmospheric Environment Service, Environment Canada, the United States National Atmospheric Deposition Network/ Mercury Sub-network (USNADP/MDN), the State of Maine, the State of New Hampshire and the Commonwealth of Massachusetts. This project will provide data on wet and dry deposition of mercury into the Gulf of Maine Airshed and will also provide at the end of five years an assessment of mercury deposition into the Gulf of Maine.

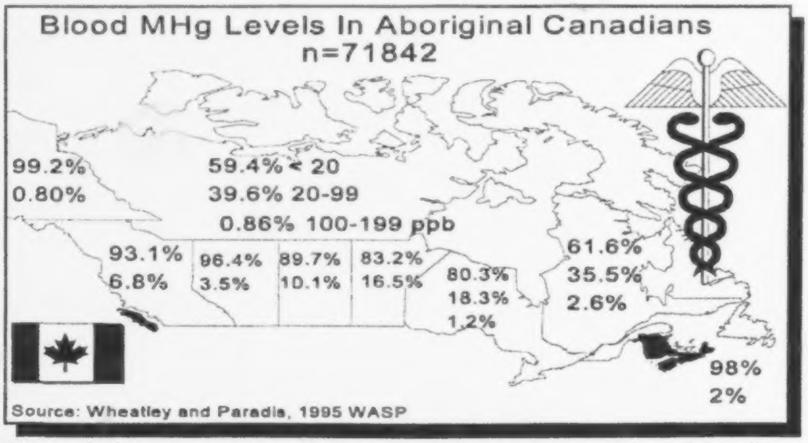
Sediment profiles show elevated levels of mercury in the Canadian arctic. European sources are thought to play a significant role in mercury contamination of the northern circumpolar regions including arctic Canada (Figure 5). It is also thought that arctic regions act as sinks for mercury because it is not as easily revolatilized back to the atmosphere.

RISK ASSESSMENT

Human Health

In Canada, the northern aboriginal peoples depend on fish as a major part of their diet. Thus, contaminants in fish and marine mammals are of special concern to this group. Hydro projects are very large in some areas of northern Canada, and flooding increases methylation and bioaccumulation of mercury in fish that frequent these impoundments. For example, in northern Quebec mercury in 700 mm pike before impoundment in 1978 averaged 0.6 ppm and in 1988 averaged 3 ppm (Dumont 1995). People that depend on these fish are considered a group that is potentially more at risk than the average Canadian. Thus, studies have traditionally focused on these northern communities. It is unclear where the inorganic mercury originates from. Some geologists suggest that building damns on bedrock that contains higher levels of mercury will result in more bioaccumulation in fish. Other scientists have suggested that the mercury has originated from atmospheric sources and as the soils and vegetation are flooded, the inorganic mercury is methylated. With the concern for global mercury contamination, health studies may now need to be considered for other subsidence fish eating groups.

Wheatley and Paradis, 1995 reported mercury blood levels in aboriginal people from 514 communities across Canada.(Fig .6) Twenty three percent had blood methyl mercury levels greater than 20 ug/l which is the



Source: Wheatley and Paradis, 1995 WASP

Figure 6. Mercury concentrations in the blood of aboriginal Canadians (Data from: Wheatley and Paradis, 1995).

World Health Organization (WHO) assessment level and 1.6 % had blood concentrations above 100 ug/l, which is the WHO benchmark for people at risk. The largest percentage of cases with greater than 100 ppb in the blood were from Quebec, Ontario, the NWT and Manitoba. The provinces of Quebec, Ontario, Manitoba and New Brunswick carry health advisories for fish consumption. Although mercury levels are above guidelines in selected innu and inuit foods in northern areas of Canada, the nutritional value of these foods are very important and cutting them out of their diet would be more detrimental in the short term than allowing them to eat food with mercury levels above 0.5 ppm. Health Canada is considering lifting bans on consumption of certain natural foods in Northern Canada. It is difficult to show with confidence any health effects of chronic mercury exposure. Most of the information in the literature is based on high level exposures.

An epidemiological study in New Zealand was designed to evaluate the neurological effects of prenatal methyl mercury exposure in children (Gearhart et al. 1995). Six year old children whose mothers had been exposed to methyl mercury from fish consumption were given performance test for academic attainment, language development, fine and gross motor coordination, intelligence and social adjustment. It was suggested from the New Zealand studies that the No Adverse Effect Level (NOAEL) for future children was when maternal hair contained 17 ppm or less. The WHO now suggests 6 ppm of mercury in hair as a guideline. The New Zealand study supported the present methyl mercury dietary intake NOAEL reference dose of 0.3 ug/kg/day and suggested that the intake guideline did not need to be lowered for fetal protection as was suggested by some studies. A Lowest Observable Adverse Effect Level (LOAEL) of 3 ug/kg/day was developed from the Iraq mercury poisoning incident. To obtain the NOAEL, an uncertainty factor of 10 is sometimes applied to the LOAEL. The WHO has in the past shown that a daily intake of 0.48 ug/kg of total mercury would not show any observable effect. ATSDR, 1994 suggested a minimal risk level (MRL) of 7 ug/kg/day of inorganic mercury which is much less toxic than the organic forms.

In reality all food, drink and inhalation sources have to be assessed to determine total contaminant intake. Richardson, et al. 1995 estimated the average adult Canadian takes in 0.11 ug/kg of mercury/ body weight per day. His study showed that for the general population dental amalgams accounted for a greater amount of total mercury absorbed into the body than by fish consumption. Health Canada is presently reviewing the safety of mercury dental amalgams. Groups or individuals eating a regular diet of fish (subsistence fishers) of the right trophic level would likely be more at risk from mercury poisoning than other groups exposed to inorganic mercury because mercury in fish is ninety percent organic which when consumed is much more toxic to warm blooded animals than inorganic forms.

Product	Concentration mg/kg FW	Average mg/kg
A. Tuna Tin	0.09-0.14	0.12
B. Tuna Tin	0.22-0.31	0.26
C. Tuna Tin	0.02-0.11	0.07
D. Tuna Tin	0.05-0.06	0.05
Shark Fillet	0.12-0.14	0.13

Figure 7. Mercury concentrations in selected commercial products (Pilgrim, 1994).

Fish is the primary source of methyl mercury intake to animals and humans. Not all fish bioaccumulate mercury to the same level and marine species contain higher selenium that counters mercury toxicity. Consumption guidelines such as those used by Ontario are the most practical as a public advisory because it suggests the types of fish that may or may not be eaten and shows spatial distribution of contamination. Mercury levels found in selected species (pike, bass, perch etc) may be above the guideline of 0.5 ppm but if not consumed on a regular basis may be safer to public health than those fish containing levels below the guideline but consumed in larger quantities. Daily, weekly and monthly methyl mercury intakes are more meaningful estimates of risk than a guideline. It is more practical to ensure that fish species eaten on a regular basis does not exceed the LOAEL for methyl mercury as defined for humans. For example, assuming a 17 kilogram child consumes 10 grams of fish containing 0.26 ppm of mercury, the daily intake would be 0.15 ug/kgbw/day which is below the reference dose of 0.3 ug/kg/day (Figure 7). However, aboriginal subsistence fishers eat as much as 200 grams of fish per day. An average women weighs 60 kilograms which at 0.5 ppm of mercury in the fish translates into 2.5 ug/kgbw/day. You can see where the concerns for pregnant woman arise based on existing LOAELs.

Wildlife Health

There are a variety of fish eating birds and mammals that bioaccumulate mercury and may carry body burdens that are considered a hazard to their health by laboratory standards. However, some populations may have always had higher mercury concentrations than others. The spatial trends in mercury concentrations that different populations exhibit are important in understanding the mercury issue. For example, it was shown that belugas and ringed seals from the eastern arctic had lower levels of mercury than belugas and ringed seals from the western arctic and was suggested related to the geology (Wagemann 1995). The mercury concentrations in sediments also showed a similar pattern. The 0.5 ppm guideline for mercury in fish is established for human protection. However, due to the amount of fish consumed per body weight, concentrations of mercury lower than 0.5 ppm in the diet of piscivorous birds have been shown to produce reproductive and behavioural affects (Scheuhammer 1995). The disadvantage of some wildlife toxicological studies is that extrapolations from one species to another are used. For example, doves may be tested in the laboratory and the effects suggested to be the same for loons. Even the same avian species tested in the laboratory may react differently than free living species. The role of parameters such as selenium in detoxification of methyl mercury also needs to be more clearly defined.

Piscivorous species in regions with higher mercury deposition, geological anomalies and also those in areas that favour methylation, such as partially acidified watersheds or watersheds with large wetlands high in DOC (Rudd, pers. comm.) are more likely most at risk from mercury contamination. Species such as yellow perch and bass occupy a different trophic level and have a greater ability to accumulate mercury (Figure 8). Usually piscivorous species that feed on a tropic level fish that bioccumulate the most mercury (higher BAF) will be most at risk. For example, loons feeding on yellow perch will accumulate more mercury than mergansers feeding on salmon parr.

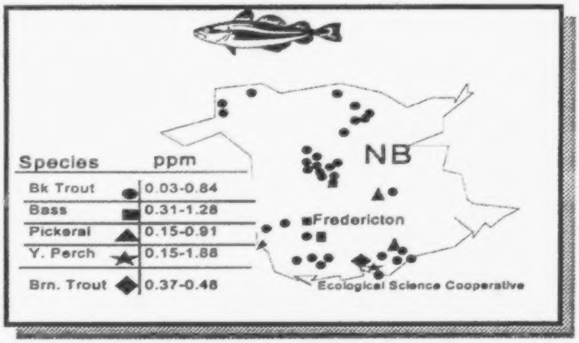


Figure 8. Mercury content of fish (fillets ppm ww) in New Brunswick 1994.

Body burdens of mercury can be good indicators of the health of fish eating mammals and birds. Mercury is excreted in the feathers of birds or hair of mammals thus are good indicators of the concentrations in other body organs. Monteiro and Furness, 1995 suggested seabirds to be good indicators of mercury pollution, concentrations in feathers are typically below 10-15 ppm ww, liver 10-15 ppm dw, and eggs 2 ppm dw. Eisler, 1987 suggested 5 ppm ww as a benchmark in feathers protective of birds. Between 1984-1990, 221 Common Loons were collected in Minnesota and examined for mercury contamination (Ensor et al. 1992). Of the 221 examined in that study, 128 were found dead or dying and 93 were live captured. Mercury concentrations in the feathers ranged between 0.08-83 ppm ww with a geometric mean of 3.6 ppm ww. Twenty two percent had mercury concentrations in the liver above 13 ppm ww which is considered a benchmark associated with impaired reproduction. The mean mercury concentration in feathers was 8.67 for 41 adults examined with a high of 29 ppm, and 2.66 ppm for 91 juveniles examined. Meyer et al. 1995 examined the mercury content of blood and feathers from 35 adult loons in Wisconsin. The study showed that loons from lakes with low ANC had higher blood levels of mercury. In a recent study in Atlantic Canada, mercury concentrations in loons were found to be the highest yet observed in North America (Burgess, 1996). Burgess reported a mean near 6 ppm in the blood of some adult loons from Atlantic Canada. Mercury blood concentrations range between 0.5-2.0 ppm for Northern American loons. Wider scale studies and effects on population productivity now need to be determined.

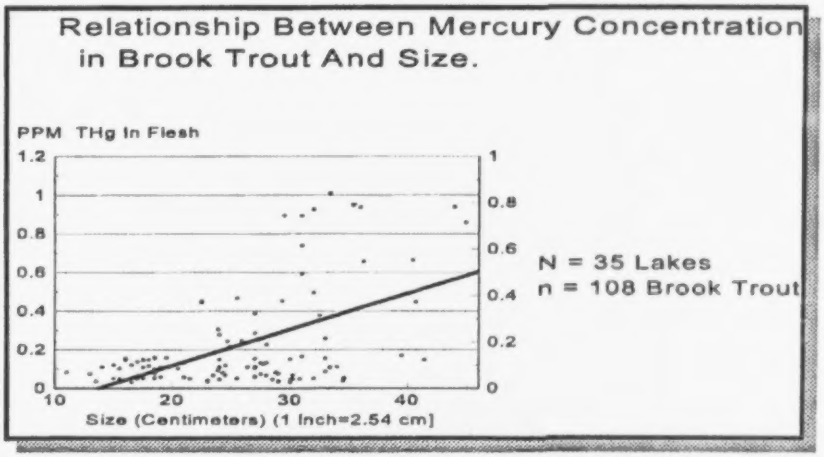


Figure 9. Mercury in brook trout from various water bodies in New Brunswick, 1994.

A survey of the mercury content of primarily brook trout from New Brunswick was conducted in 1994 and a health advisory similar to that used by the State of Maine was issued. Although brook trout was the primary species examined, a limited number of other species, smallmouth bass, pickerel, yellow perch and brown trout were also collected and analysed for mercury (Tables 1-5). Yellow perch is a primary food source for loons but loons will take brook trout or what ever is available on their breeding lakes. Loons prefer prey 20 cm or less thus for brook trout this corresponds to mercury concentration of less than 0.20 ppm (Figure 9). As seen from the regression line size was not the only factor affecting bioaccumulation of mercury. There is considerable variability in mercury concentrations amongst brook trout between the 30-40 cm range.

Conclusions

Heavy metal protocols cannot realistically be implemented for mercury in Canada until there is more geographically linked information which can substantiate the extent of mercury contamination. Multi-media (air, sediments and biota) temporal and spatial trends in mercury concentrations need to be established. A mercury deposition network is essential to evaluate anthropogenic loading. To understand the mercury cycle, linkages with regional and global networks are essential.

Sediment core profiles suggest that eastern Canada and arctic Canada are ecosystems impacted by airborne mercury. Because of the association between mercury uptake and acidification, piscivorous birds and mammals from the Atlantic region of Canada, which is very acid sensitive should be further assessed for mercury impacts. Although selected wildlife populations may have higher body burdens of mercury than others, the effects are unclear. There is considerable variation in the literature on the lowest observable effect of mercury on piscivorous species. Most toxicological tests have been designed to assay acute conditions and chronic effects because they are more difficult to determine are not as clearly defined.

Aboriginal groups in northern Canada and subsistence fishers are potentially more at risk from mercury exposure than groups that do not depend on fish as their main food. The large reservoirs in northern Quebec and other areas in Canada contribute to elevated mercury in fish and groups living in this area should continue to be monitored. However, as the mercury issue unravels, other risk groups may be identified. For subsitence fishers the nutritional value has to be weighed against the ban to limit the consumption of fish. In many areas where natural diets are more important than store bought foods, the nutritional need may out weigh the concern for contaminants in that food and advisories will be lifted.

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Table 1. Mercury in skin off flesh of brook trout from New Brunswick, 1994.

Lake	County	Total mercury in skin off flesh (ppm)	Length (cm)
1. A	Charlotte	0.55	36.3
		0.18	24.9
		0.38	29.3
2. Beaver	Victoria	0.04	17.5
		0.05	18.5
		0.05	17.0
3. Beaver Brook	York	0.12	17.0
		0.13	18.5
		0.10	16.5
4. Bennett	Albert	0.07	11.0
		0.06	13.0
		0.10	18.0
5. Britt Brook	Victoria	0.06	18.5
		0.13	27.0
		0.24	27.0
6. Caldwell	Restigouche	0.09	17.5
7. California	Northumberland	0.04	32.0
		0.13	33.0
		0.09	34.0
		0.14	39.5
8. Caribou	Restigouche	0.19	28.0
		0.74	31.0
		0.77	32.0
9. Catamaran	Northumberland	0.07	33.0
		0.09	33.4
		0.12	41.4

Table 2. Mercury in skin off flesh of brook trout from New Brunswick, 1994.

10. Cheaver	Victoria	0.03	16.5
		0.05	17.0
		0.04	15.5
		0.04	17.0
11. Deer Lake	York	0.09	27.0
		0.04	21.5
		0.09	27.0
12. Flood	Kings	0.12	16.0
		0.13	18.5
		0.12	18.0
13. Goodwin	Nortumberland	0.09	23.9
		0.03	24.3
		0.04	30.8
14. Grants	Northumberland	0.09	20.5
		0.03	13.5
		0.04	15.0
		0.11	28.0
		0.10	24.5
		0.10	24.0
15. Half Moon	Northumberland	0.04	30.0
		0.04	28.5
		0.03	29.0
16. Island	Victoria	0.11	27.5
		0.08	27.0
		0.08	19.0
17. Jenkins	Victoria	0.13	17.5
		0.13	16.0
		0.13	19.5
18. Kenny	Northumberland	0.03	30.0
		0.03	34.5
		0.05	34.6

Table 3. Mercury in skin off flesh of brook trout from New Brunswick, 1994.

19. Long	Victoria	0.232 0.323 0.414	24.0 27.0 32.0
20. Louis	Northumberland	0.062 0.041 0.057	27.5 30.2 30.2
21. Mains	Northumberland	0.13 0.39 0.75	24.0 25.5 29.5
22. McFadden	Saint John	0.07 0.11 0.09	28.8 27.7 26.4
23. Middle Peaked Mountain	Northumberland	0.13 0.14 0.22	30.0 31.0 33.0
24. Moose	York	0.04 0.06 0.06	24.0 23.5 24.5
25. Neary Pond	Victoria	0.06 0.06 0.06	29.0 28.0 27.0
26. Risteen Deadwater	York	0.80 0.84 0.45	35.4 33.4 31.0
27. Scoullar	Charlotte	0.37 0.31	40.7 32.5
28. Sisson	Victoria	0.09 0.38 0.09	14.0 22.5 15.5

Table 4. Mercury in skin off flesh of brook trout from New Brunswick, 1994.

29. South Oromocto	Charlotte	0.78	43.9
		0.55	40.4
		0.72	44.9
30. States	Restigouche	0.03	23.0
		0.04	25.5
		0.03	23.0
31. Third	Madawaska	0.05	21.0
		0.10	15.0
		0.04	17.0
32. Trousers	Victoria	0.37	22.5
		0.79	35.5
		0.62	31.0
		0.78	36.0
33. Trout	Queens	0.25	23.9
		0.20	24.7
		0.21	25.9
34. Wild Goose	Restigouche	0.04	15.5
		0.05	19.0
		0.05	17.5
35. Yellow	Victoria	0.12	17.5
		0.08	18.5
		0.11	21.0

Table 5. Mercury in fish from New Brunswick, 1994.

Lake	Species	County	Total mercury (ppm)	Length (cm)
36. Cassidy	PICKERAL	Kings	0.14 0.13	32.5 28.0
37. Harvey	SMALL MOUTH BASS	York	0.36 0.31 0.75	35.5 32.6 44.4
38.Lake Stream	PICKERAL	Queens	0.91 0.47	43.0 40.0
	YELLOW PERCH		0.15	18.5
39. Loch Lomond	BROWN TROUT	Saint John	0.37 0.48	38.5 49.0
	YELLOW PERCH		1.88	29.0
40. Mactaquac	SMALL MOUTH BASS	York	1.38 0.88 0.90	43.0 45.0 35.0
33. Trout	LANDLOCKED SALMON	Queens	0.34 0.25	37.7 28.2
27. Scoullar	WHITE SUCKER	Charlotte	0.15 0.21	35.9 33.0

FLUXES OF MERCURY IN LG-2 RESERVOIR BEFORE AND AFTER FLOODING.

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The purpose of this study was to estimate the temporal fluxes of mercury among the diverse biotic components of the aquatic ecosystem of the LG-2 reservoir. Fish were grouped into prey species and predators. Data on fish were compiled to permit estimation of standing stocks and total Hg burdens. Biomass fluxes through these compartments were calculated by bioenergetics, and Hg fluxes were then calculated using the appropriate concentrations. A model of plankton dynamics was used to estimate biomass fluxes through phyto- and zooplankton, and Hg fluxes were estimated using appropriate concentrations. Hg release from decomposing vegetation and soils and subsequent methylation in the water column were estimated using a model based on experimental data. For each compartment, the necessary fluxes were calculated to explain observed biomass and Hg quantities. For predatory fish, the amounts, both of biomass and Hg available from measured prey species, were insufficient to explain quantities found, explaining only 12% and 6%, respectively. For measured prey species, plankton could only account for 45% and 7% of biomass and Hg, respectively. Quantities of methyl mercury resulting from decomposition of flooded vegetation and soils were more than sufficient to explain the quantities in biota. These observations lead to the following conclusions: [1] there is a quantitatively large but unmeasured fish stock being exploited by predators, and this stock has higher Hg levels than measured prey; [2] both measured and unmeasured prey stocks are exploiting a food source other than plankton, most likely benthos, which is quantitatively greater in both biomass and Hg fluxes than plankton.

Because of the magnitude of the discrepancies involved, these conclusions are valid in spite of uncertainties in the calculations. These conclusions are also consistent with recent observations on fish stomach contents and on Hg levels in benthos. Unfortunately, there is no quantitative data on benthic biomass as this proved impossible to measure quantitatively after reservoir flooding.

CHANGES IN TOTAL MERCURY IN TISSUES OF HARBOUR PORPOISES, *Phocoena phocoena* (L.) FROM THE BAY OF FUNDY, CANADA, DURING 1970-1989.

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Levels of total mercury (Hg) were measured in liver, kidney and muscle tissue of harbour porpoises (*Phocoena phocoena* L.) collected from the Bay of Fundy in 1989 (n=50) and compared to Hg levels recorded for porpoises from this region in 1970-72 (n=43). Total Hg was significantly lower ($p<0.05$) in all three tissues of females, and kidney tissue of males sampled in 1989, possibly reflecting a decrease in anthropogenic input of Hg and a change of oceanographic conditions in the western North Atlantic documented for this time. The more obvious reduction in females may be related to the disburdening of Hg through lactation and through transplacental transfer during pregnancy.

MERCURY IN MARITIME RIVERS AND ON THE SCOTIAN SHELF.

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DFO's Marine Chemistry Division at the Bedford Institute of Oceanography is studying the concentrations of inorganic contaminants in 29 rivers to monitor the river source of contaminants into the coastal zone. Last fall and during this year's field season, samples were also collected for the analysis of "Total" Hg. These samples were oxidized with BrCl and UV light in a clean bench and analyzed using gold preconcentration Cold Vapour Atomic Fluorescence Spectroscopy. The details of the analysis method and data for total Hg from the river surveys will be presented.

The levels of "reactive" Hg on the Scotian Shelf have also been measured on two oceanographic expeditions as part of programs designed to monitor the concentrations of metal contamination in the water column on the Scotian Shelf. The data presented from both expeditions will describe the levels of Hg on the Scotian Shelf.

MERCURY FLUXES IN THE ST. LAWRENCE BASIN.

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Mercury distribution and speciation have been determined in the waters and sediments of the Saint-Lawrence Basin. Recent results obtained in a monitoring programme started in spring 1995 indicate that concentrations in the St. Lawrence River waters range from 1 and 3 pM and from 0.3-0.6 µmol/kg for the dissolved and particulate phases, respectively. These concentration levels are among the lowest measured in natural waters. A few speciation measurements reveal that the monomethylmercury account for less than 5% of the total mercury. In unfiltered samples of water from the Estuary and Gulf the concentrations are also in the picomolar range, with the highest levels associated with surface and bottom turbid waters. Conversely, riverine and estuarine sediments exhibit relatively high concentrations, probably in relation with anthropogenic inputs from the previous decades. A mass balance calculations at the scale of the whole system (Gulf, Estuary and River) show that [1] the atmospheric deposition largely exceeds the riverine inputs, [2] the atmospheric deposition exceeds the elemental mercury evasion to the atmosphere, and [3] that the Atlantic Ocean is a significant source for the System. This environment seems to act as a cleaning system by scavenging mercury from different sources.

BILAN MASSIQUE DU MERCURE DANS LE BASSIN DU SAINT-LAURENT

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SOMMAIRE

Une forte contamination du bassin du Saint-Laurent par le mercure a été mise en évidence depuis déjà deux décennies en particulier par l'étude de la distribution de ce métal dans les sédiments (ex.: Loring, 1975). Les profils verticaux de distribution des concentrations en mercure, accompagnés par des estimations de l'âge des dépôts sédimentaires, indiquent généralement la superposition de deux types d'apport de mercure anthropique. D'une part, un apport qui va régulièrement croissant depuis le début du 20ième siècle et, d'autre part, un apport beaucoup plus considérable qui apparaît dès la fin de la Deuxième Guerre mondiale, culmine au tournant des années 70 et tend à s'effacer de nos jours. Ce patron de distribution est clairement apparent à l'examen de carottes sédimentaires à la source du Saint-Laurent [Lac Ontario (Eadie *et al.*, 1983)], et dans la région Montréalaise [Lac Saint-Louis (Rukavina *et al.*, 1990)]. Dans l'état actuel des connaissances sur les variations des émissions anthropiques de mercure à l'échelle de ce bassin (Cossa, 1990) comme à celle de l'ensemble de l'hémisphère nord (Mason *et al.*, 1994), ce type de distribution en mercure dans les sédiments constitue la trace de l'augmentation continue depuis le début de l'ère industrielle de l'apport atmosphérique par transport à longue distance auquel se sont surimposés, à partir des années 1950, d'importants rejets directes dans les eaux, qui ont été considérablement réduits suite aux réglementations édictées au début des années 70.

Dans les sédiments du Chenal Laurentien cette interprétation est moins manifeste, du fait en particulier du lissage des profils par la bioperturbation (Gobeil et Cossa, 1993). Alors que les concentrations en mercure

dans les sédiments de l'Estuaire Maritime sont en décroissance depuis vingt ans, une diminution parallèle des concentrations en mercure des sédiments du Golfe n'est pas évidente. La progressive inversion de l'importance relative de l'apport fluviatile par rapport à l'apport atmosphérique de l'estuaire vers le Golfe serait à l'origine de ces variations dans la structure des profils de la tête `du Chenal jusqu'au Détroit de Cabot. Le présent travail se propose, grâce à l'utilisation des techniques analytiques qui permettent aujourd'hui la mesure du mercure dans les eaux (ex.: Cossa *et al.*, 1994), d'estimer les apports fluviatiles, et de les comparer avec les échanges atmosphériques et l'accumulation sédimentaire. Le bilan massique du mercure dans le Système Fleuve-Estuaire- Golfe permet de vérifier l'hypothèse posée.

Dans le cadre du « Plan d'action Saint-Laurent Vision 2000 » un suivi hebdomadaire des concentrations en mercure dissous et particulaire dans les eaux du fleuve Saint-Laurent à Québec a été mis en place au printemps 1995. Les résultats obtenus jusqu'en août 1995 font état de concentrations allant de 1 à 3 pM pour la phase dissoute et de 0.3 à 0.6 nmol g^{-1} pour la phase particulaire. Ces concentrations, qui ne situent pas le Saint-Laurent dans la liste des fleuves les plus contaminés du monde industrialisé (Cossa *et al.*, sous presse), permettent une estimation des apports fluviatiles annuels moyens en mercure dissous et particulaire vers l'Estuaire respectivement à 0.4 et 2.4 kmoles. Par ailleurs, sur la base de données de la littérature établies pour des régions voisines (Glass *et al.*, 1991 ; Burke *et al.*, 1995), ainsi qu'à partir de résultats de modèles (Mason *et al.*, 1994 ; Shannon et Voldner, 1995), on peut estimer le dépôt atmosphérique sur la vallée du Saint-Laurent autour de 50 nmol m^{-2} a^{-1}. L'évasion de mercure élémentaire volatile (Hg°) des eaux de surface vers l'atmosphère est, quant à lui, estimé à environ 0.09 nmol m^{-2} j^{-1}. Ce dernier flux est calculé à partir de mesures simultanées des concentrations en Hg° dans les eaux de surface et l'atmosphère de l'Estuaire Maritime (Cossa et Gobeil, données non publiées recueillies en 1992) selon le modèle d'échange gazeux utilisé par Fitzgerald *et al.* (1994). Enfin l'accumulation sédimentaire actuelle en mercure, calculée sur la base des résultats de Gobeil et Cossa (1993), se chiffre à 5.2 kmol a^{-1} pour l'Estuaire Maritime et 7.3 kmol a^{-1} pour le Golfe. Le bilan de masse en mercure suggère que non seulement l'ensemble de l'apport fluviatile du Saint-Laurent est accumulé dans l'Estuaire Maritime, mais encore qu'une partie non négligeable du mercure sédimenté dans cette zone provient de l'atmosphère probablement *via* les eaux du Golfe apportées par la circulation estuarienne. Corrélativement, le mercure accumulé dans les sédiments du Golfe du Saint-Laurent est d'origine essentiellement atmosphérique.

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A REVIEW OF SENSITIVE ANALYTICAL METHODS FOR TOTAL AND METHYL MERCURY.

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This paper is a review of the methods currently being used in a number of labs to measure low levels of total and methyl Hg in biota and water. These techniques have been developed to permit determinations of low ng/L to pg/L concentrations in water and low μg/kg levels in biota. Discussion covers both sample preparation and Hg detection. The most important sources of reagent blanks are identified, and reagent screening, preparation and purification are discussed. For both biota and water, digestion procedures are compared for total Hg. Methods for carrier gas drying of total Hg are compared. For MeHg, distillation is compared to solvent extraction. Preconcentration methods for total Hg in water and for MeHg in both water and biota are examined. Advantages and disadvantages of the aqueous-phase ethylation procedure for MeHg are discussed. For detection, CVAAS and CVAFS are discussed and compared, as well as GC-ECD for MeHgCl.

SEDIMENT TOXICITY
SESSION CHAIR: K. JOP

MFO INDUCTION IN FISH EXPOSED TO CONTAMINATED SEDIMENTS FROM THE ST. LAWRENCE RIVER NEAR MASSENA, NY.

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Sediments in the St. Lawrence River near Massena, NY are highly contaminated with PCBs, PAHs, aluminum, cyanide and fluoride due to the operation of several aluminum industries. As a `Superfund' site, the river may be dredged to remove these sediments, and the potential exists for their wider dispersion downstream. To supplement `before dredging' bioassays of sediment effects on growth and mortality of fish and invertebrates, we measured induction of mixed function oxygenase (MFO) enzymes in rainbow trout exposed to dilutions of whole sediments, sediments kept in suspension by vigorous aeration, sediment elutriates, and methanol extracts of freeze-dried sediments. In all cases, MFO induction increased in an exposure-dependent fashion to 100-200 fold higher than control activity. Induction was evident at concentrations as low as 0.002-0.02% sediment, and mortality occurred at the highest concentrations (2-20%). Reference sediments caused either no induction (Long Point Bay, Lake Erie) or low levels of induction (St. Lawrence River). The nature of the compounds causing induction is unknown, although some congeners of PCBs and some PAHs are known to be both inducers and highly toxic. These results suggest that sediment-borne contaminants are bioavailable, bioactive, and could be released and dispersed during dredging.

CHRONIC EXPOSURE TO ENVIRONMENTALLY CONTAMINATED BOTTOM SEDIMENTS REDUCES SPERM QUALITY IN AMERICAN PLAICE (*Hippoglossoides platessoides*).

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The Baie des Anglais area (Baie Comeau) on the lower St. Lawrence estuary is characterized by inputs of industrial (pulp mill and aluminum smelting) and domestic effluents and, consequently, the bottom sediments are some of the most contaminated in the St. Lawrence River system. Levels of polycyclic aromatic hydrocarbons are reported to exceed 10 mg/kg dried bottom sediment from this area, relative to <1 mg/kg in the open estuary. The objective of the present study was to determine whether reproduction is compromised in male American plaice living within these sediments. The fish were experimentally exposed in the laboratory to sediments, with varying degrees of environmental contamination, obtained by bottom sampling from Baie des Anglais. The exposure period was 5 mo in duration, immediately preceding the normal spawning period (June). Semen was collected from all mature males and used to fertilize eggs from a non-sediment exposed female held under laboratory conditions for 2 yr. Males exposed to bottom sediments containing the highest degree of contamination had a significantly ($p<0.05$) lower capacity for fertilization (84%), relative to non-sediment exposed fish (100%). Other parameters, such as sperm number and motility, were not significantly different between groups. The relationship between semen quality and molecular exposure markers (metalothionein and cytochrome P450 1A1) will be discussed.

CHRONIC EFFECTS OF PESTICIDE EXPOSURE IN SEDIMENT TO THE MARINE POLYCHAETE *Neanthes arenaceodentata.*

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Organochlorinated pesticides persist in the environment, particularly in sediments. Organisms exposed to such compounds are likely to suffer chronic rather than acute effects. Thus, acute toxicity tests are unlikely to truly assess their potential impact. A full-life cycle ("third generation") 120 d toxicity test was designed to assess the impact of organochlorinated pesticide exposure to the marine polychaete *Neanthes arenaceodentata*. A two tiered approach was used: Tier I involved reference sediment spiked with a range of concentrations of a pesticide mixture bracketing the concentrations found in natural sediments; Tier II involved field sediments collected from a coastal area impacted with high concentrations of the same pesticide mixture. Testing measured a number of endpoints, including survival, growth and reproduction. Survival and growth were unaffected in either tier by any of the test sediments. Reproductive endpoints, however, were depressed in both tiers relative to the reference sediment. This mode of action (i.e. observed effects) is in accord with mammalian and avian studies and, similarly, could result in significant population level effects.

ENVIRONMENTAL ASSESSMENT OF THE COMPOUNDS FROM CREOSOTE-TREATED PILINGS IN MARINE SEDIMENTS.

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Creosote, a complex chemical mixture has been widely used as wood preservative. Creosote contains a number of organic components, some of which are biologically active. The chemical composition of creosote is critical to its fate and effects in environment. Due to differential partitioning of these components, however, estimates of the environmental impact of creosote and creosote-treated wood cannot be solely based on the composition of creosote. Constituents of creosote leached out from treated wood are partitioned between the water column and sediment. Partitioning is based on a variety of factors including water solubility, actinal/water and organic carbon partition coefficients. The current study focuses on the characterization of the constituents of the creosote, the partitioning of these constituents between surface water sheen, water column and sediment and assessing the impact of these constituents deposited in sediment and sediment pore water. The analytical measurements were performed with neat material, used to preserve treated pilings from the site which the environmental samples were collected. Five areas of investigation comprising the evaluation of sediment samples were: [1] evaluation of environmental conditions around existing creosote pilings; [2] evaluation of environmental conditions around restoration of pilings; [3] assessing creosote migration into the surrounding environment 1 yr after pile-driving; [4] confirmation of creosote sediment toxicity in laboratory studies; and [5] evaluation of photoinduced toxicity on test organisms exposed to PAHs. Pore water samples were evaluated using the short-term chronic exposure with *Mysidopsis bahia*, while bulk sediment samples were evaluated with 10 d sediment toxicity tests with *Ampelisca abdita*. Verification of organism response and analyses of laboratory-spiked samples was performed using the reference sediment collected at Moss Landing Harbour, Moss Landing, California. Evaluations were also performed to determine the effects of photoinduced toxicity on *A. abdita* exposed to creosote-spiked sediment. Test organisms were exposed to normal fluorescent lights and UV fluorescent lights that simulate the natural UV spectrum of sunlight. The biological and analytical results of the field and laboratory exposures are being used to evaluate and determine risk of creosote-treated pilings on the benthic marine ecosystem.

BIOMARKERS OF POLLUTION
SESSION CO-CHAIRS: S.C. COURTNEY and D. CYR

DOSE AND TIME RESPONSE OF CYP1A mRNA INDUCTION AND CLEARANCE IN CHEMICALLY TREATED ATLANTIC TOMCOD (*Microgadus tomcod*).

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The enzyme cytochrome P4501A1 (CYP1A1) has been proposed as a sensitive biomarker of exposure to certain organic contaminants including coplanar PCBs, dioxins and furans, and PAHs. Atlantic tomcod (*Microgadus tomcod*) from industrialized estuaries have been shown to have higher CYP1A mRNA levels than tomcod from undeveloped estuaries. As part of a biomonitoring program of organic contaminants in Gulf of St. Lawrence estuaries, CYP1A mRNA levels are measured in tomcod semi-annually. In order to calibrate this response, levels of CYP1A mRNA were quantified in Atlantic tomcod intra-peritoneally injected with different concentrations of a dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin or TCDD), a coplanar PCB (3,3',4,4'-tetrachlorobiphenyl or TCB), and two PAHs benzo[*a*]pyrene (B[*a*]P) and beta naphthoflavone or BNF). Additionally, the rates of CYP1A mRNA induction and clearance were quantified in Atlantic tomcod intraperitoneally injected with single doses of these chemicals and sacrificed at times ranging up to 72 d. Dose-responsive CYP1A mRNA induction was elicted by all four chemicals. Kinetic profiles of CYP1A mRNA induction and clearance differed among chemicals. For example, maximum induction of CYP1A mRNA occurred 72 h after treatment with B[*a*]P, but not for at least 25 d after treatment with TCB and TCDD. These results suggest that the CYP1A system of Atlantic tomcod responds to organic contaminants of environmental concern, at environmentally relevant concentrations, and that profiles of CYP1A1 induction and clearance may provide valuable information regarding the identity of the inducing chemical.

A DIET OF SAND SHRIMP *(Crangon septemspinosa*) FROM THE MIRAMICHI RIVER INDUCES CYTOCHROME P4501A mRNA IN THE ATLANTIC TOMCOD (*Microgadus tomcod*).

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Induction of Cytochrome P4501A (CYP1A) in fish has been associated with effluent from pulp and paper mills. Atlantic tomcod (*Microgadus tomcod*, Walbaum) from the Miramichi River, N.B., have been shown to have significantly higher levels of CYP1A mRNA than do tomcod from less industrialized rivers. Induction of CYP1A mRNA is not only found in tomcod close to a pulp mill on the Miramichi (i.e. within the 1% effluent plume), but also has been shown in tomcod from the tidal estuary many kilometers downstream. It is likely that sediment-bound contaminants are the source of inducing agents for tomcod well out in the estuary. It is of interest, both from a scientific and an ameliorative point of view, to determine the route of exposure for tomcod in the estuary. Diet seemed a likely candidate for a route of exposure, since the majority of the diet of tomcod consists of benthic or epibenthic organisms, and these types of organisms have been shown to accumulate and concentrate some sediment-bound contaminants. On a seasonal basis, the most important food item (in terms of biomass) for Miramichi tomcod is the sand shrimp *Crangon septemspinosa* (Say). We fed groups of tomcod *Crangon* collected from either the Miramichi River, or the Kouchibouquac River (an unindustrialized reference river). After one month of these diets, we compared hepatic CYP1A mRNA levels in these two sets of fish. We found significantly higher CYP1A levels in the tomcod fed the Miramichi *Crangon*.

ETHOXYRESORUFIN-O-DEETHYLASE (EROD) INDUCTION IN RAINBOW TROUT EXPOSED TO DILUTED OIL SAND WASTEWATER.

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Toxic industrial wastewaters must be assessed for their potential sublethal effects before they can be safely disposed in the environment. Ethoxyresorufin-*O*-deethylase induction was assessed as a potential bioindicator of stress in rainbow trout (*Oncorhynchus mykiss*) exposed to sublethal concentrations of oil sands tailings water. The mixed-function oxygenase system in trout responded rapidly following a definable concentration-response relationship; however, it proved to be a relatively insensitive indicator of sublethal exposure to oil sands tailings water. Increased activity and maximal induction occurred rapidly within 24 h of exposure to 0.3 and 0.6 times the LC$_{50}$(Toxic g/kg ß-naphthoflavone (*i.p.*). We present data that suggests that the different levels of induction observed in trout exposed to tailings water vs those injected with ß-naphthoflavone may be indicative of two different P450 isoforms, the CYP4A1 isoform responding to organic acidic surfactants in oil sands tailings water and the CYP1A1 isoform, the isoform generally associated with most xenobiotic transformation in fish, responding to ß-naphthoflavone.

SEMIPERMEABLE MEMBRANE DEVICES (SPMDs) ACCUMULATE INDUCERS OF FISH MFO FROM PULP MILL AND OIL REFINERY EFFLUENTS.

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Semipermeable Membrane Devices (SPMDs) are tubes of leaflet polyethylene, containing a thin film of triolein. Neutral organic compounds with K$_{ow}$ >1 and diameters < 10Å pass through the membrane pores and are dissolved in the lipid. Dialyses of the tubes provides a time-integrated sample that can be used in bioassays, or more commonly, used for chemical analysis. SPMDs deployed for 14 da in waters of the Athabasca River and in effluents from four pulp mills and one oil sands mining and refining facility accumulated chemicals that induced mixed function oxygenase (MFO) in a fish cell line, *Poeciliopsis lucida* hepatic carcinoma (PLHC-1). Dose-response curves for MFO induction in cells exposed to SPMD-extracts were compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Extracts of SPMDs from three of the four pulp mills were two to five times as potent (62.0, 53.5, and 29.7 pg TCDD-EQ/g) as extracts of SPMDs exposed to background river water (average 12.6 pg TCDD-EQ/g). SPMDs exposed to effluent from a fourth mill had potencies within the 95% confidence interval of background. The concentrations of MFO inducers in SPMDs exposed to river water increased and was highly variable downstream of Fort McMurray (58.5-728 pg TCDD-EQ/g), suggesting input from natural erosion of the oil sands. SPMDs deployed in effluent from the oil refinery accumulated the most MFO-inducing chemicals (16,800 pg TCDD-EQ/g). Although this study was preliminary, the results suggest the four pulp mill effluents contributed small quantities of MFO inducers to the Athabasca River. By contrast, very high quantities of MFO inducers were discharged by the oil refinery effluent.

TIERED BIOINDICATORS TO DETERMINE BIOLOGICAL EFFECTS OF BLEACHED KRAFT PULP MILL EFFLUENT ON MINK (*Mustela vison*).

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Semi-aquatic predators such as mink are exposed to anthropogenic contaminants directly through the water column or by bioaccumulation of compounds through the food chain. The effect of bleached kraft mill effluent (BME) on mink (*Mistelle Vinson*) was studied using a tiered approach, beginning with clinical, biochemical, hematological, reproductive and pathological effects. Hepatic enzyme induction was measured by Ethoxyresorufin-*O*-deethylase (EROD) activity. Xenobiotics may modulate immune function, thereby affecting competitive fitness or disease resistance in exposed populations. The immunotoxic potential of BME to cell mediated and humoral immunity was investigated in these mink. Sampling and testing were carried out on live animals using relatively noninvasive techniques. Peripheral blood mononuclear cells (PBMC) were cultured with various mitogens and proliferation was measured. Delayed type hypersensitivity (DTH) response, an integrated function of cell mediated immunity, was assessed. This response requires intact communication among antigen-presenting cells, T lymphocytes and macrophages. Humoral, or, antibody mediated immunity was measured after an enzyme-linked immunosorbent assay (ELISA) for antibody detection was developed for mink.

There were no differences in the physiological, reproductive or pathological variables examined between BME-exposed and control mink. There was no difference in PBMC proliferation between groups. The hepatic EROD activity was 1.8 times greater in exposed females and 2.0 times greater in exposed males compared with controls. The DTH response was impaired in the BME-exposed mink based upon skin thickness measurements, histopathological assessment and image analyzer technology. The antibody levels in the exposed mink were higher than in controls indicating that BME modulates the humoral immune response in mink.

ASSESSMENT OF THE GENOTOXIC POTENTIAL OF PULP MILL EFFLUENT USING BACTERIAL, FISH AND MAMMALIAN ASSAYS.

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Extracts of bleached kraft mill effluent (BME) have been shown to contain mutagenic activity and to induce biochemical responses in fish, such as increased activity of the mixed-function oxygenase (MFO) enzyme system. Recent work has shown that Ames-positive substances in BME are direct-acting mutagens, and that the majority of mutagenic activity is extracted by X'D-4 and X'D-8 resins and eluted by aqueous solutions of sodium hydroxide (NaOH) or methanol (MeOH). In this study, the genotoxic potency of BME extracts was further assessed using a bacterial (Umu-C) genotoxicity assay, an *in vivo* hepatic micronucleus assay with trout, and an *in vitro* assay for DNA strand breaks with Chinese hamster lung V79 cells and two mouse liver cell lines (TIB 73 and TIB 75). The majority of mutagenic substances from the filtered effluent were absorbed by X'D-4 and X'D-8 resins and DEAE cellulose, and were effectively removed from these adsorbents by NaOH or MeOH. The mutagenic fraction extracted by X'D-4 and eluted by MeOH consistently induced DNA strand breaks in the assays with V79, TIB 73, and TIB 75 cells. Activity of these fractions was not altered by the addition of rat liver microsomes or dialysed liver cytosol from various vertebrate species. The fractions extracted by X'D-8 and eluted with NaOH, and extracted by X'D-8 and eluted with MeOH did not induce DNA

damage in assays with V79 cells. The genotoxic responses indicated direct acting genotoxicity to mammalian and fish cells and correlates with bacterial mutagenicity, and that these bioassays are complementary when applied to screening of BME samples for genotoxic potential.

GENETIC EFFECTS OF CONTAMINANT EXPOSURE ON WILDLIFE POPULATIONS: A REVIEW OF POTENTIAL METHODS OF ASSESSMENT.

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This review aims to identify the potential risks to wildlife as a consequence of exposure to genotoxins and to identify the techniques most useful in assessing these risks. These evaluations are complicated because contaminant exposure acts both to restructure naturally occurring genetic diversity and, when contaminants have mutagenic activity, to enhance the rate of introduction of new variation. Contaminant exposure often leads to genetic change in natural populations. Short-lived organisms can develop resistance to contaminants, with only modest impacts on diversity in the rest of the genome. Resistance is, however, less likely to evolve in species with small population sizes, such as many wildlife species. Such species will experience population declines or extinction as the impact of contaminants on physiological systems which is not counteracted by gene replacements. Even when adaptation to exposure occurs, populations may suffer diminished fitness as a consequence of the mutagenic effects of contaminants. These effects range from an increase in the incidence of developmental abnormalities to shifts in chromosomal and gene structure. The assessment of this broad range of impacts can only be accomplished with a spectrum of analytical approaches. However, recent advances in molecular and developmental genetics are now making possible the detailed assessment of these mutagenic impacts in natural populations.

IN VITRO ASSESSMENT OF THE GENOTOXICITY OF BENZO[*a*]PYRENE.

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The mutagenic effects of benzo[*a*]pyrene, a proven genotoxin, on calf thymus, zebra mussel and other DNA isolates was evaluated using lectrokinetic assessment and evaluation by image analysis and densitometry. DNA was exposed to concentrations of the genotoxin with and without enzyme activation. In some instances restriction enzymatic cleavage was carried out. The resulting restriction fragments were then separated and characterized by agarose and acrylamide electrophoresis. The comparison of fragments of exposed genetic material with similar fragments from unexposed DNA demonstrates the resulting genetic damage. The results are presented and discussed.

STATISTICS FOR ESTIMATING POTENCY FROM NON-QUANTAL DATA
SESSION CO-CHAIRS: P.V. HODSON and J.L. PARROTT

ESTIMATING POTENCIES OF CYTOCHROME P4501A INDUCERS IN CELL CULTURES

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Induction of cytochrome P4501A (CYP1A) is a well known biochemical response of vertebrate animals exposed to planar halogenated aromatic hydrocarbons (HAHs) such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and some polychlorinated biphenyl (PCB) congeners. Hepatoma cell lines and primary hepatocyte cultures have been particularly useful for studying the mechanism of CYP1A induction and for determining dose-dependent effects of HAHs and other compounds (e.g. polyaromatic hydrocarbons, photooxidized amino acids and pulp mill effluents) on CYP1A. CYP1A catalytic activity is easily estimated using the ethoxyresorufin-*O*-deethylase (EROD) assay, and relative potencies of compounds can be obtained from dose-response curves. One practical application of EROD analysis in cultured cells is for use as a bioassay for estimating the toxic potency of complex mixtures of compounds extracted from environmental samples (e.g. animal tissues, soil and water). Potencies are usually expressed relative to TCDD in units of TCDD-equivalents (TCDD-EQs). Although new methods for preparing cell cultures and for measuring EROD activity developed recently in this laboratory allow one to easily obtain dose-response curves, there is some uncertainty on how to compare potencies of inducers because [1] not all inducers elicit the same maximal EROD response, [2] dose-response curves are not always parallel and [3] there are some situations where EROD activity does not accurately reflect the amount of catalyst (CYP1A protein) present in the cells. This paper describes the influence that these factors have on potency estimates and makes suggestions on how results from CYP1A-based bioassays should be used and interpreted.

DOSE-RESPONSE CURVES FOR EROD INDUCTION IN FISH: WHERE DO WE COMPARE POTENCIES?

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Dose-response relationships were examined in detail for the induction of ethoxyresorufin-*O*-deethylase (EROD) activity in rainbow trout by 5 polychlorinated dibenzo-*p*-dioxins (PCDDs) and 4 polychlorinated dibenzofurans (PCDFs). For some congeners, slopes of EROD activity versus exposure to PCDD or PCDF were not parallel to slopes for TCDD; for PCDDs, slopes usually decreased with increasing chlorination. Because of the different slopes, the potencies of PCDDs and PCDFs relative to TCDD decreased with increasing dosages; hence, toxic equivalent factors (TEFs) varied with the endpoint selected. TEFs were usually largest (congeners were closer in potency to 2,3,7,8-TCDD) when potencies were compared at threshold doses of PCDD/F, where EROD activity first increased above control levels. TEFs derived from conventional ED_{50}s based on non-transformed activities were smaller because non-parallel curves diverged. Because EROD activity increased logarithmically in response to doses of PCDD/Fs, the midpoint of the regression, where confidence limits are smallest, was the log ED_{50}. Comparisons of log ED_{50}s gave TEFs between the threshold and ED_{50}-derived TEFs. Dose-response curves relating EROD activity of small rainbow trout exposed to increasing concentrations of chemicals in water or to industrial effluents also resulted in different slopes and different maximum activities; some compounds caused much greater activity than others. Problems with assessing these data are similar to those encountered for comparisons of potencies of PCDDs and PCDFs, but with the added consideration of the differing maxima. Derivation of estimates of potency require endpoints and analyses that are both statistically and biologically sensible.

AN INTRODUCTION TO THRESHOLD MODELLING OF NON-QUANTAL BIOASSAY DATA

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Abstract

The comparison of potencies among toxicants causing sub-lethal responses, is plagued with problems such as choice of endpoint, and variations in shape and slope of the dose-response curve. One ecologically relevant endpoint is the threshold concentration, or the concentration at which the response being measured is different from that of a control. Threshold models are a class of models containing this endpoint as a parameter. The utility of the hockey-stick model as a threshold model is illustrated and algorithms for parameter estimation are reviewed.

Introduction

The estimation of an 'environmentally safe' dose is a problem that toxicologists have been grappling with for years. For the case of non-quantal bioassay data, two general approaches are popular.

The first approach estimates the largest toxicant concentration that does not produce a response significantly different from that of a control. This is known as the 'no observed effect concentration (NOEC)', The lowest observed effect concentration is a similar endpoint and is defined as the lowest concentration producing an effect significantly different from that of the control. Some problems associated with estimating environmentally safe doses using this approach are that the endpoints (NOEC and LOEC) are restricted to being one of the concentrations used in the experiment, the endpoints increase with increasing variability, and the endpoints decrease with decreasing sample size. Thus the estimated NOEC or LOEC can be manipulated through choice of sample size, and exposure concentrations or by altering the precision with which the experiment is conducted. Stephan and Rogers, (1985) and Hoekstra and Van Ewijk, (1993) discuss problems associated with these endpoints.

The second class of methods involves predicting the concentration associated with a specific degree of change in response, relative to that of the control. The minimum degree of change necessary to protect the environment is a matter of some debate. In an effort to make this decision less critical and to provide some measure of safety for the environment, the lower bound of the 95% confidence interval of the concentration causing a p% change in response is often chosen as the environmentally safe concentration. Another problem with this approach is that the choice of model corresponding to the observed dose response may not be obvious. Also, the predicted concentration may be quite sensitive to choice of model if an extreme quantile is chosen.

Threshold models are a class of parametric models that contain a form of the environmentally safe dose as a model parameter. This is the threshold concentration and can be defined as the concentration below which no measurable response is seen. Note that this also defines the LOEC. Threshold models presume that a threshold exists for a specific organism/toxicant concentration. This is contrary to the more traditonal approaches used in aquatic toxicity testing where models such as probit models for quantal data presume a continuum in response, down to infinitesimally small doses. A brief argument for existence of thresholds follows.

A toxicant mediates its effect on an organism at a specific biochemical receptor. The failure of this receptor is generally an all or nothing response. However an organism has an ability to correct minor internal imbalances in a variety of ways and the blockage or failure of a small number of receptors may not greatly affect the organism. As the concentration of the toxicant reaches a critical or threshold level, the organism is no longer able to function normally, and some degree of response, correlated with dose, is seen. One exception to this paradigm occurs when a substance has the ability to damage DNA. In this case a single alteration, can potentially trigger a whole organsim response.

Although the concept of a threshold is theoretically plausible, a clearly defined threshold may not occur in practice. This may be due to the nature of the endpoint being measured, with responses measured at higher levels of biological organization exhibiting poorly defined thresholds. For example, growth is an integrative measure of a complex series of biochemical reactions. The integration of effect(s) at perhaps, multiple sites of toxic action may obscure the threshold. This, coupled with variations in individuals may result in an apparently gradual increase in response with dose. Dinman (1972) offers a particularly interesting argument for the existence of thresholds.

Threshold models have been successfully used in human toxicology to study the effects of SO_2 on the prevalence of chronic bronchitis (Yanagimoto and Yamamoto, 1979), and, lead and heme synthesis, (Piomelli, et al, 1982) and in phytotoxicology to study the effects of metals on root growth in wheat (Taylor et al, 1990). The use of threshold models in aquatic toxicology seems to be largely ignored.

Threshold Models for Non Quantal Responses

The hockey stick model, broken stick model or segmented regression model consists of two (or more) lines joined at a point of intersection, or join point. In our terminology the join point corresponds to the threshold dose. For threshold modelling the first line is a constant, a_1, and is known as the background response.

The model can be written as:

$$\begin{aligned} f(x) &= a_1 && for\, x \leq x_o \\ &= a_2 \pm b_2 x && for\, x > x_o \end{aligned}$$

where x_0 is the threshold value and a_1 is a control or background response. The response f(x), may be a continuous or discrete random variable. We consider the the case where the response is continuous. Two forms of the threshold model follow.

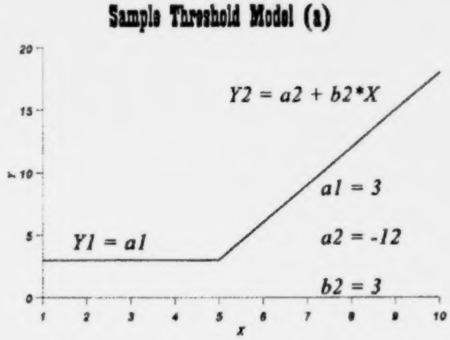

Figure 1a. A sample threshold model where the response increases after the threshold.

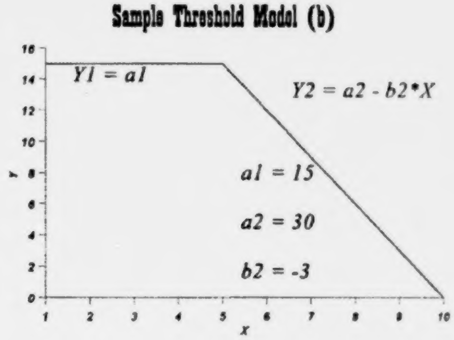

Figure 1b. A sample threshold model where the response decreases after the threshold.

Estimating Model Parameters

Parameters can be estimated using simple linear regression routines if we cast the independent variable into an alternate format using dummy variables. Dummy variables are constructed variables which retain relevant portions of the data structure and allow some deterministic structure to be imparted to the regression. In the case of threshold models we wish to impart a constant value to the response corresponding to the background response rate, and a monotonically increasing or decreasing function corresponding to the dose response. The methods used in estimating parameters varies depending on how much information we have regarding the independent variables. We discuss methods for parameterizing the threshold model in three cases, but first introduce an example which will be used to illustrate the methods used to estimate model parameters.

This data is extracted from Yanigimoto and Yamamoto, (1979). It describes the number of cases of bronchitis per approximately 2000 residents from each of 8 control areas and 9 polluted areas for a total of (approximately) 34,000 people. The independent variable is the average concentration of SO_2 during 3 years. A plot of the data follows.

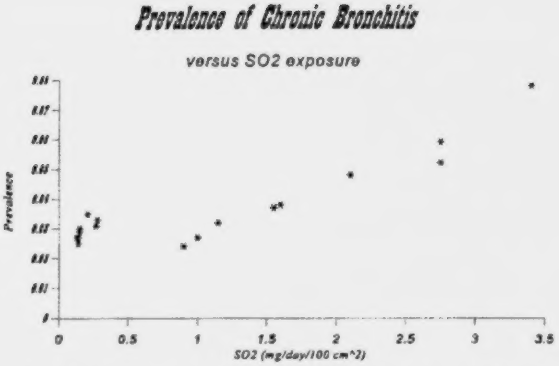

Figure 2. Scatter plot of example data from Yanigmoto and Yamamoto (1979).

In the first case, Case 1, we know which points belong to each model and know the value of the join point or threshold. This is clearly not the case for aquatic toxicity testing , but serves as an introduction to model parameterization.

We can fit a hockey-stick model when the join point is known and the spacing of the independent variable is even, by using dummy variables to parameterize the regression model. We assume for the time being that the join point occurs at (0.032,1.15) and parameterize the model as follows.

Order the data by the independent variable, which for this example is SO_2. Then, we reacast the information held in the independent variable SO_2 concentration, into two dummy variables. The following pseudocode may be used when the the independent variables are equally or unequally spaced.

```
Difference = X at Join Point - Minimum X;

 If X  < X at Join Point then:        Dummy 1 = X- Minimum X,
                                      Dummy 2 = 0.

If X > X at Join Point then:          Dummy1 = Difference,
                                      Dummy2 = X - X at Join Point.

If if X = X at Join then:    Dummy1 = Difference,
                                      Dummy2 = 0.
```

An example of the parameterization when the join point is known, follows.

Table 1. Sample Parameterization When Join Point is Known

SO_2	Prevalence	Dummy1	Dummy2
0.13	0.027	0.00	0.00
0.14	0.027	0.01	0.00
0.14	0.025	0.01	0.00
0.15	0.03	0.02	0.00
0.15	0.029	0.02	0.00
0.21	0.035	0.08	0.00
0.27	0.031	0.14	0.00
0.28	0.033	0.15	0.00
0.9	0.024	0.77	0.00
1	0.027	0.87	0.00
1.15	0.032	1.02	0.00
1.55	0.037	1.02	0.40
1.6	0.038	1.02	0.45
2.1	0.048	1.02	0.95
2.75	0.059	1.02	1.60
2.75	0.052	1.02	1.60
3.4	0.078	1.02	2.25

We can fit the following model using a regression package.

$$Y = a_1 + b_1 * \text{dummy1} + b_2 * \text{dummy2}$$

Test the hypothesis that $b_1 = 0$. This tests the assumption that a threshold or a constant response exists prior to the threshold value. For this data set we obtain $b_1 = 0.01150$, with s.e. = 0.002580 and a t-value of -0.45 with an associated p-value of 0.6629 testing the null hypothesis: $b_1 = 0$. Thus, a true threshold exists.

We know that the threshold occurs when the value of $SO_2 = 1.15$. This occurs at intercept = 0.029375, with s.e. = 0.001449 and a t-value of 20.27 with an associated p-value of 0.0001, testing the hypothesis: intercept = 0. The threshold value is not 0.

Our interpretation is that the background prevalence of chronic bronchitis is 2.94 %. The prevalence increases if SO_2 levels rise above the threshold value of 1.15 (mg/day/100 cm^2). A plot of the predicted value versus the observed values gives a visual indication of the model fit.

In the second case, Case 2, we know which points belong to each model but the join point is not known. This is true for the sample data set since we know that 8 of the points were obtained from the control area and 9 from the polluted area.

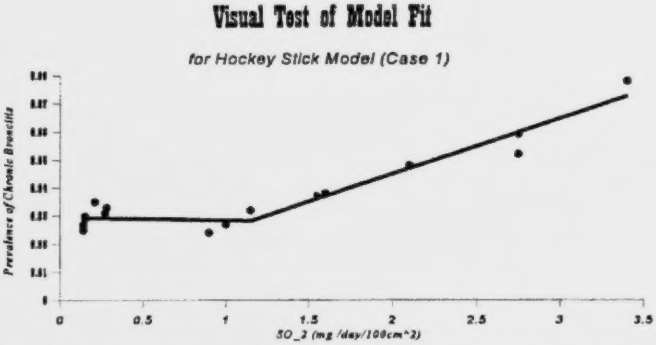

Figure 3 Figure 3: A visual test of fit for the hockey stick model in the case where we know which points fall on each model segment and the join point or threshold is known.

If the join point is not known, but it is known which points belong to each segment we proceed as before with the construction of dummy variables. However another dummy variable is required to estimate the point of intersection. Using the previous pseudocode we make the following additions.

```
If X  < X at Join Point
  then  Dummy 3 = 0,
else, Dummy 3 = 1.
```

Now fit the model,

$$Y = a_1 + b_1 * \text{dummy1} + b_2 * \text{dummy2} + b_3 * \text{dummy3}$$

When this is done we obtain the following parameter estimates.

Table 2. Parameter estimates for hockey stick model , case 2.

Parameter	Estimate
Intercept	0.0296221918
B_1	-0.0039719410
B_2	0.0188683351
B_3	0.0040298409

The background prevalence of bronchitis is now 2.96%. The threshold SO_2 level can be estimated as follows.

We know that for the first line

$$\text{Predicted } Y = 0.0296 + -0.003972 \text{ Dummy}_1 \,.$$

Due to the parameterization for the second line, $\text{Dummy}_1 = 1.02$ at the point of intersection, therefore the equation for the second line is given by:

$$\text{Predicted } Y = 0.02962 - 0.003971{*}(1.02) + 0.01887{*}\text{Dummy}_2 + 0.004030, \text{ or}$$
$$\text{Predicted } Y = \text{Intercept} + b_1{*}(\text{Difference}) + b_2{*}\text{Dummy}_2 + b_3$$

We know that at the intercept, $\text{Dummy}_2 = \text{Dummy}_1 - 1.02$. If we substitute this into the previous equation we have two equations with the unknown, Dummy_1. We solve this to obtain $\text{Dummy}_1 = 0.80642$. This is converted to the original scale by the equation $\text{Dummy}_1 = X$ - Mininum Value of X. We obtain a threshold of 0.936 mg SO_2 (mg/day/100 cm^2).

In the third case, Case 3, we do not know which points correspond to each portion of model, nor do we know the threshold concentration. The easiest way to solve this problem is to place restrictions on the parameters and use a nonlinear regression package to solve the resulting problem. Consider the following two equations comprising the hockey-stick model.

$$Y_1 = a_1$$
$$Y_2 = a_2 + b_2 X$$

At the join point, X_0, $Y_1 = Y_2$, therefore a1= a2 + b2*x0. We use this constraint in the following pseudocode to fit the threshold model.

```
If X < threshold
  then model y = a1
else model y = a2 + b2*x.
```

We guess at the initial value for the threshold and let the program iterate to the solution. This can be run on any nonlinear program that allows the user to constrain the variables and does not require user-specified derivatives. Otherwise restrictions on the derivatives are also required.

The model is fit and the following parameter estimates are obtained. a2 = 0.004958, b2 = 0.01995, and the threshold of 0.291 occurs until SO_2 levels exceed 1.2099 mg/day/100 cm^2. A visual test of model fit follows.

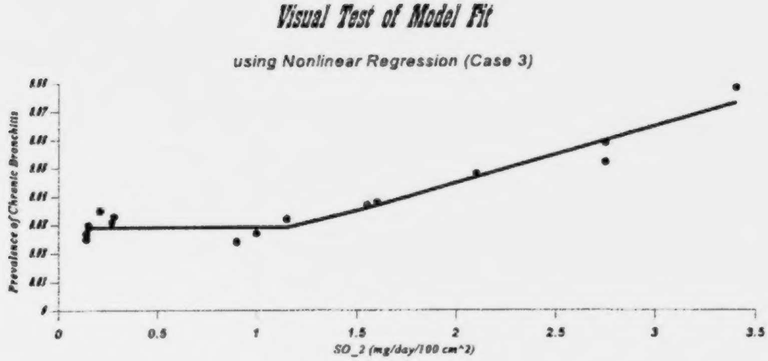

Figure 4 A visual test of fit for the hockey-stick model when the it is not known which points fall on each line, and the value of the threshold is unknown.

Discussion:

Threshold models, while possessing the intuitively appealing aspect of containing an estimate of the environmentally safe dose, are subject to some problems. Threshold models, especially those where the dose response above the threshold is not linear, may be difficult to fit. As discussed in the introduction, a threshold may not exist for a particular toxicant/effluent - species combination.

The design of experiments intended to estimate thresholds, would require an initial range-finding test to determine the approximate location of the threshold. Then, at least 2 concentrations, must be chosen to fall within **each** phase of the threshold. More complicated models would require more concentrations to estimate the increased number of parameters.

In addition, the usual problems associated with non-linear modelling apply to fitting threshold models when using non-linear optimization. These include problems with convergence, model misspecification, uniqueness of parameter values and the use of assymptotic standard errors.

In conclusion, we have found that simple threshold models are easily fit and are useful in avoiding problems associated with estimation of the no-observed effect concentration. They also do not require specifying the quantile that defines an ecologically relevant effect as in a regression type model. While experiments designed to estimate threshold models may be more costly to run, there is an increased confidence in our estimation of environmentally safe exposures. The decision to obtain a more costly, but improved estimate of an environmentally safe exposure is ultimately a function of the value we place on our environment.

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NON-LINEAR REGRESSION MODELS FOR QUANTITATIVE TOXICITY TESTS

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For estimation of the inhibition concentration (ICp) in non-quantal toxicity tests, analysis using non-linear regression techniques is a promising approach. However, choice of an acceptable model is a challenge; hormesis (stimulation at low concentrations) adds complications to the estimation problem. Some alternative models which permit estimation of the ICp, together with confidence limits, will be presented, together with application to some data.

ESTIMATING RELATIVE POTENCY WITH NON-PARALLEL RESPONSE CURVES

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A method is proposed that permits the estimation of the relative potency based on two non-parallel response curves for non-quantal endpoints in aquatic toxicity experiments. Under certain conditions, the dose (or log dose) which first causes a 50% of the maximum activity can be the basis of the relative potency. Valid standard errors can be derived such that meaningful statements can be made about the assessment of the risk of the compound to the environment. A review study and a comparative study will also be included.

ESTIMATING RELATIVE TOXIC POTENCY: THREE APPROACHES FOR DEALING WITH NON-PARALLEL DOSE-RESPONSE CURVES

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Estimating the relative potency of single chemicals or mixtures is often complicated by non-parallel dose-response curves. Two materials may have the same potency at one dose; for example, they may have the same LD_{50}, but have very different potencies at other doses. The observed relative potencies of substances also depends on the effect under study: use of different end points may lead to different estimates of relative potency.

This presentation will explore three methods used to assess toxic potency in mixtures and single substances: Toxic Equivalent Quantities (TEQs), the reference dose (RfD) and the benchmark dose (BD). The TEQ method is used to evaluate the potency of a mixture of related compounds, and has been applied to complex mixtures of chlorinated dioxins and dibenzofurans. The RfD is used to estimate a safe daily dose for long-term exposure of humans (or animals) to single chemicals. The benchmark dose represents a daily dose which is expected to produce adverse effects in a small fraction of exposed people (or animals). Both TEQs and the RfD are currently used in risk assessments in Canada and the U.S., while the BD is currently being developed as a risk assessment tool. The advantages of each approach will be discussed and compared. Limitations in the science of obtaining, applying and interpreting these quantitative estimates of toxic potency will also be discussed.

CONSIDERATIONS IN THE DEVELOPMENT AND APPLICATION OF TEFs FOR RISK ASSESSMENT OF MIXTURES

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Typically for risk assessment purposes the general assumption of additivity of chemical mixtures prevails based on the observation of additivity at exposures less than threshold levels for individual chemicals (Krewski *et al.*, 1989; Stara *et al.*, 1987; NAS, 1983). The concept of toxic equivalency factors (TEFs) for the assessment of mixtures has been exploited for dioxin-like chemicals, specifically *2,3,7,8*-chlorine substituted dioxins and furans and the *non-ortho* and *mono-ortho* polychlorinated biphenyls. The scientific validity of the TEF approach is based on a number of underlying assumptions regarding the additivity of the individual chemical constituents with respect to the parallelism of the dose-response curves, similarities between the predicted dose range and the test dose range and exposure periods, similarities in the toxicokinetics of the individual chemical constituents and the species sensitivities of the toxic endpoints for which the TEFs are derived and applied (Neubert *et al.*, 1992).

It is becoming clear that repeated exposure of laboratory mammals to mixtures of PCBs and PCDD/Fs may not induce toxicity in the way that would be predicted based solely on data from acute, single chemical exposures and it is expected that a similar situation exists for aquatic organisms chronically exposed to mixtures. Data suggest that the interaction of mixtures of PCBs and PCDD/Fs may generally be antagonistic rather that additive, and it is likely that alterations in toxicokinetics caused by exposure to mixtures of these chemicals and by repeated dosing play a role in the non-additive toxicity of mixtures. These findings lead to the conclusion that TEFs derived from short-term bioassays using single chemical exposures cannot be used reliably in risk assessment involving multiple chemical and/or repeated exposure, and would most likely lead to an overestimate of risk.

Current understanding points to the need to consider the following in developing and applying TEFs for risk assessment purposes: 1) toxicokinetics and metabolism; 2) dosing regimes used for generating laboratory data; 3) interactions with Ah receptor; 4) role of Ah receptor and biological relevance of endpoints upon which TEFs are based.

With respect to influences of toxicokinetics and metabolism on the biological effects of mixtures of similar chemicals that are co-administered, several mechanisms can explain non-additive toxicity.
Similar chemicals may compete for binding at the Ah receptor (Nagao *et al.*, 1993; Darnerud *et al.*, 1993; Pohl and Holler, 1995; Rao and Unger, 1995), absorption of mixture components may be slower compared to individual doses (Nagao *et al.*, 1993; Rozman *et al.*, 1995), metabolism may be speeded up (McKinley *et al.*, 1993) or receptor production increased (Darnerud *et al.*, 1993) as a result of enzyme induction caused by other components of the mixture, excretion rate may be increased leading to faster excretion and lower toxicity (McKinley *et al.*, 1993). Examples of the non-additive effect of mixtures include the following: The experimentally determined teratogenic potential of 2,3,4,7,8-TCDF plus PCDD mixture was lower than predicted using TEFs (Nagao *et al.*, 1993). Tumour promoting activity of PCDD mixtures in a rat liver bioassay was lower than predicted from TEFs (Schrenk *et al.*, 1994). The immunosuppressive effects of PCB and PCDD mixtures were lower than predicted using TEFs (Harper *et al.*, 1995). In a rare report of synergy between PCBs given in a mixture, non-ortho dioxin-like PCBs and less potent ortho substituted PCBs produced enhanced induction of EROD in rat hepatocytes over that which would have been expected based on individual chemical induction potencies. In the same study a mixture of only non-ortho PCBs showed perfect additivity (Schmitz *et al.*, 1995).

Repeated dosing, another factor which must be considered in the application of TEFs for practical use, may enhance metabolism and excretion resulting in lower effect than single dose (Soontornchat *et al.*, 1994; Pohjanvirta and Tuomisto, 1994). In addition, differences in half lives among chemicals may result in time differences to reach steady state, so short term TEFs may not reflect longer term relative differences in tissue concentration and hence toxicity (DeVito and Birnbaum, 1995; Van den Berg *et al.*, 1994)

In addition to considering kinetic factors, the role of the Ah receptor and the mechanism of action must be taken into account when assessing the applicability of TEFs to conditions other than those under which they were specifically developed. The Ah receptor hypothesis that is widely accepted is that the PCB or PCDD/F binds to the receptor, the receptor complex is translocated to nucleus where it binds to another protein (arnt), the complex then binds to specific regions of DNA resulting in gene regulation, including production of mRNAs coding for P450 synthesis and production of protein products result in toxicity (see Matsumura, 1994). A second mechanism, involving protein phosphorylation appears to operate as well (Matsumura, 1994 - review; Enan and Matsumura, 1995). This mechanism involves Ah receptor binding, but not AHH induction. In this case the PCB or PCDD binds to the receptor, the receptor complex influences protein kinase activity, gene regulation is influenced by protein kinases and both protein products and phosphorylation products cause toxicity. Key evidence for this mechanism comes from the observation that a protein kinase inhibitor can prevent some manifestations of TCDD toxicity *in vivo* in mice (Bombick and Matsumura, 1987) and that protein kinase stimulation by TCDD has been demonstrated in nucleus-free system, demonstrating lack of need for nuclear translocation (Enan and Matsumura, 1995). This influence on protein phosphorylation could result in changes in glucose metabolism, leading to widespread disruption of homeostasis and explaining the wide array of toxic effects. Various aspects of the phosphorylation mechanism have been discussed by Muralidhara *et al.*, 1995; Wang and Safe, 1994; Weber *et al.*, 1994; Hatsumura *et al.*, 1994; Enan and Matsumura, 1994; Oguri *et al.*, 1993; Olsen *et al.*, 1994; and Ryu *et al.*, 1995.

Another important issue that must be considered in assessing the applicability of TEFs is the biological relevance of Ah enzyme induction mechanism-based TEFs. TEFs currently in use are based on premise that relative differences in toxic potency of PCBs and PCDDs are reflected in relative potency of enzyme (AHH) induction. Mounting evidence that some toxicity is not associated with Ah induction sheds doubt on the reliability of TEFs derived based on Ah induction. The following examples illustrate this point. Methylcholanthrene induces the same genes at same degree of induction as does TCDD, but does not cause the same spectrum of toxicity and is much less toxic (Riddick *et al.*, 1994; Pohjanvirta and Tuomisto, 1994). In a comparison of Ah receptor mRNA content in various tissues between TCDD-sensitive and TCDD-resistant mice, there were no differences between strains (Li *et al.*, 1994). The dose-response for TCDD-induced liver tumour promotion is different from dose-response for P450 gene expression such that the liver tumour dose-response cannot be predicted from enzyme induction data (Maronpot *et al.*, 1993). PCB congeners with different affinities for Ah receptor were tested for their ability to affect PMN function *in vitro*; the one with high affinity did not influence PMN function, while the one with low affinity did. This may indicate that immunotoxicity of PCBs is not related to Ah induction (Ganey *et al.*, 1993). Also, Ah induction was not correlated with immunotoxicity in human peripheral blood lymphocytes *in vitro* (Lang *et al.*, 1994). On the other hand, immunosuppression induced by several PCBs, and PCDD/Fs in mouse splenocytes was correlated with Ah receptor binding affinity (Harper *et al.*, 1995). The characteristics of Ah receptors in various species are similar, although there is a wide variation in TCDD susceptibility (Pohnjavirta and Tuomisto, 1994). These examples provide a clear indication that TEFs based on AHH induction would not be predictive of relative potencies for a number of toxic endpoints,and therefore cannot be broadly applied.

Several new approaches to TEF development have been reported, although it is not yet clear whether they lead to more or less applicable TEFs than those currently in use. For PCBs, affinity for the Ah receptor is governed by several physicochemical parameters. These have been used by a computer model to predict relative toxicities. This is still based on receptor binding affinity, but minimizes the need for animal testing in identifying possibly hazardous PCB-like chemicals (Kafafi *et al.*, 1993). EROD induction in rat hepatoma cells has been suggested as basis for potency comparison among PCDD/Fs. This is based on a QSAR model developed based on molecular and physical chemical descriptors for predicting TEFs, again without the need for whole animal testing (Tysklind *et al.*, 1994). The WHO/IPCS consensus approach (Ahlborg *et al.*, 1994), was based on a weight of evidence approach that considered as much relevant data (from experimental animals) as was available. The strengths of this approach are that all relevant data were considered without limitation to specific endpoints, repeated dose *in vivo* data were used where possible and consensus among a group of experts was involved. Weaknesses of this approach are that the database was not consistent for all chemicals, making comparisons difficult, it would be difficult to include all congeners using this approach, due to lack of data, and TEFs derived from laboratory animal and *in vitro* data may not be suitable for use in body burden assessment or for ecotoxicology assessment.

A review of the relative toxic potencies of PCDDs/PCDFs and coplanar PCBs to aquatic and avian species revealed that considerable differences in species sentivities and toxic endpoints exist (Van den Berg *et al.*, 1995). Thus, TEFs derived from mammalian studies would likely over- or under- estimate the toxic potency of individual and mixtures of PCDDs/PCDFs and PCBs to avian and aquatic species. Furthermore, given the wide range in physical-chemical properties of these chemicals, the influence of exposure media and exposure pathway may play a significant role in the observed relative differences in species and congener specific sensitivities.

The major advantage of the TEF approach is its simplicity and ease of application, these same advantages may also be considered distinct disadvantages leading to the inappropriate use of TEFs, such as i) direct application of TEFs to analytical data for soils and sediments without consideration of factors governing fate, transport, uptake and distribution by biological organisms; ii) estimation of toxic potency of body residues based on the incorrect assumption that all detectable"dioxin" is available to the Ah-receptor, when the majority is lipid bound; iii) comparison of TEQs to an exposure limit toxicologically unrelated to the toxic endpoint on which the TEFs are based. For the purpose of ecological risk assessment species-specific and endpoint-specific TEFs derived using environmentally representative concentrations or steady-state tissue concentrations are required for meaningful interpretation of TEQs. Current TEFs provide a comparative tool of exposure among organisms and locations; however, as presently developed TEQs do not address in absolute terms the risks to ecosystem or human health or specific causal agents. In appropriate use of TEFs may lead to the over estimation of health risks that could lead to excessive costs to governments and industry in the development and application of remediation and abatement technologies directed at minimizing perceived health risks.

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EFFECT OF CHOICE OF MODEL ON DOSE CONVERSION FOR DIOXIN-LIKE CHEMICALS

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Toxicity equivalency factors (TEFs) are frequently used to convert doses of dioxin-like chemicals into a 2,3,7,8-TCDD-equivalent dose. While more attractive than the originally proposed alternative of assuming all dioxins were as potent as TCDD, this approach can be demonstrated to be insufficiently accurate for many current conditions of practical importance. More mathematically accurate models for dose conversion may produce substantially different risk estimates. Mathematical analyses also demonstrate that the conversion factor should be a function rather than a number such as TEFs. Other effects of changing to a conversion function from a point estimate have been examined in another context. Preliminary results using potentially more accurate models that use functions support the conclusions of the theoretical analyses.

Current TEFs require that equivalent doses are the same linear proportion for all response levels. Theoretical analyses examined two conditions: parallel and non-parallel dose-response curves. Either condition led to a contradiction for most conditions examined. The exception contradicted the assumption of thresholds for non-carcinogenic effects. Initial analyses indicate that TEFs may be significantly inaccurate when most of the risk is dependent on chemicals other than TCDD or when risk results from the sum of many relatively small exposures.

Simple functions for dose conversion also allow incorporation of more toxicology data. Furthermore, data limitations discovered during development of the model suggest directions for future research to improve both estimation of the model's parameters and development of more sophisticated models.

ADVANCES IN MICRO-SCALE AQUATIC TOXICOLOGY
SESSION CO-CHAIRS: C. BLAISE, K. LEE and P.G. WELLS

VALIDATION OF A BACTERIAL EXOENZYME TEST FOR SEDIMENT TOXICITY ASSESSMENT

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Techniques to measure the activity of microbial exoenzymes in sediment have been developed and refined by DFO for use in environmental impact studies. Preliminary studies have shown that the activity of microbial exoenzymes is sensitive to a range of toxic metals and organic contaminants. However, scientific validation is required before these methods can be routinely applied within existing monitoring programs for use in regulating ocean disposal operations and to monitor recovery rates at impacted sites.

During this study, the current protocol of the bacterial exoenzyme sediment toxicity test was validated by conducting an intercalibration exercise with technical personnel unfamiliar with the method. Further validation of the test was conducted by comparison of the test results with two other standard marine sediment toxicity test methods: the sea urchin fertilization test and the toxi-chromotest. Other validation exercises included the establishment of a reference toxicant and the evaluation of the statistical rigor of the data resulting from the method. Based on the results of this study, standardized procedures, including statistical methods used to interpret the data, will be refined and established for implementation of the method as a routine regulatory test.

MICROPLATE TOXICITY TESTS WITH MICROALGAE: A REVIEW

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Small-scale biological tests, conducted at different trophic levels, have been increasingly developed and applied since the mid-1970s. The desire to protect phototrophicity, in particular, has triggered significant work to produce microplate-based phytotoxicity procedures and stimulated the present review in this field. Since 1978, close to 50 papers, originating from ten countries, have been written on miniaturized tests making use of micro-algae. Our search has shown that liquid and solid media procedures testing with unialgal cultures, as well as tests performed with batteries of unialgal cultures, have been reported in the literature. Comparative studies carried out between microplate and flask assay procedures with metals, herbicides and industrial effluents have essentially demonstrated that toxicity data generated with microtests match those of the forerunner flask procedures. Although a few types of assessment endpoints have been called upon to report toxicity data, cell growth inhibition has been more frequently employed. In terms of measurement endpoints, the IC_{50} has most often been determined. Chronic testing procedures have generally displayed greater toxicant sensitivity responses than those of acute testing procedures, but new tests and endpoints are emerging in the latter category to challenge this present finding. In short, this review clearly establishes that microplate phytotoxicity testing procedures, based on their operational ease and versatile applications, comprise valuable tools that can be profitably employed to assess the environmental hazards of discharged chemicals. Future research directions for microplate-based algal toxicity tests are also discussed herein.

CILIATED PROTOZOA AS TEST ORGANISMS IN TOXICITY ASSESSMENTS

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Ciliated protozoa are important as food for higher trophic levels and as recyclers and remineralizers of organic material in the benthic and pelagic realms of aquatic ecosystems. The characteristics of these easily cultured and rapidly growing protozoa are well known. Given the proliferation of new microbial bioassay technologies, ciliates have become useful subjects in toxicological research, and more recently, as test organisms for bioassays.

Ciliate bioassays have been developed by a number of researchers worldwide over the past 20 yr. These bioassays have used a variety of test organisms and, as is the case with other toxicological tests, a variety of lethal and sublethal indicator parameters. Among the indicator parameters are: morphology, mortality, growth, motility, respiration, genotoxicity, and chemotaxis. This paper will summarize the recent literature on bioassays using ciliates, and assesses them in terms of their practicability and feasibility for routine monitoring of environmental toxicants.

CLONING OF THE HEAT-INDUCIBLE STRESS PROTEIN (hsp70) FROM THE MARINE SPONGE *Geodia cydonium*

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The biomarker concept involves the use of biochemical, cellular and physiological parameters as screening tools in environmental monitoring. Stress proteins - such as heat shock proteins [hsp] - match many of the conditions to be ideal candidates in a biomarker strategy for environmental monitoring. Sponges [Porifera] are one of the major phyla found in the marine hard-substrate benthos, both with respect to the number of species and biomass. However it took until recently that genes, coding for stress proteins have been cloned from sponges; ubiquitin was the first one. Now we describe the isolation of the second gene, hsp70, from the marine sponge *G. cydonium;* its size is 2.3 kb. The deduced aa sequence of this hsp70 has a M_r of 72,593. The hsp70 protein is induced in response to heat stress, as demonstrated by Western blot analysis. Our results provide the first molecular evidence that hsp70 is a useful biomarker also in sponges.

It took until recently that based on molecular DNA sequence data sponges [Porifera] have been found to have evolved from the same ancenstor as other metazoa (Müller 1995). They have the basic structural elements and the signal transduction pathways present also in other multicellular organisms. Sponges are primarily filter-feeders and are not provided with effective structural defense systems. Hence, these animals must have developed powerful metabolic strategies which enable them to resist against unfavorable environmental conditions. Sponges react to environmental stress with the induction of a series of metabolic pathways, e.g. the heat shock system (Batel et al. 1993, Müller et al. 1995a), the polyphosphate turnover (Lorenz et al. 1995), the multixenobiotic resistance transport system (Müller et al. 1995b) and programmed cell death [apoptosis] (Batel et al. 1993). For the quantification of the physiological adjustments to fluctuating environments, *in vitro* cultures [regenerating sponge cubes; single cell cultures] both from freshwater and marine sponges have been introduced (Müller and Müller,1996).

Stressors of both antropogenic and natural origin have been shown to induce the synthesis of heat shock proteins [hsps] or stress proteins, in a number of organisms from bacteria, plants to mammals (Sanders 1990).

The number of hsps induced by stressors varies and their expression is both tissue and species specific. In general five families of stress proteins are found in eukaryotes; four of them are grouped according to their molecular weights as hsp90, hsp70, hsp58-60 and hsp20-30 whereas the fifth hsp is termed ubiquitin (Schlesinger et al. 1982.). The hsps are essential cell components most of them being involved in the formation of transient protein complexes (Nover 1991). They may also play key roles during cell cycle and development. For example, the members of the hsp60 and hsp70 families are ATP-binding proteins involved in the folding of nascent and denatured proteins or protein complexes [molecular chaperons] (Rothman 1989).

We have decided to determine - for the first time - the function of hsps in sponges. Findings, obtained mainly from studies engaged with vertebrates, indicate that hsps may protect cells against environmental stressors (Lindquist and Craig. 1988). As the first step we identified the ubiquitin protein and cloned the corresponding gene from the marine sponge *Geodia cydonium* (Pfeifer 1993). We also found that ubiquitin expression in *G. cydonium* is influenced by environmental stress. Furthermore, we describe that sponges may respond to temperature stress with the induction of another stress protein, the hsp70. For this study we used the fresh water sponge *Ephydatia fluviatilis.*

In the present study we cloned the sponge *G. cydonium* hsp70 and report its amino acid sequence. In addition we show that the expression of sponge hsp70 is regulated by heat stress.

Materials and Methods

Sponge

Live specimens of *Geodia cydonium* (Demospongiae) were collected near Rovinj (Croatia). Immediately after collecting from a depth of 25 m at 16°C they have been used for the experiments. One piece each of the specimens remained untreated at 16°C for the entire period, a second piece was treated for 2 hr at 26°C and subsequently for 18 hr at 16°C. Then the material was immediately frozen in liquid nitrogen until use.

Construction of *G. cydonium* cDNA library

Total RNA was extracted from sponge tissue and cDNA library was prepared as described (Pfeifer et al. 1993). *Plaque screening:* Screening of the library was performed under low stringency hybridization using the human hsp70 cDNA [L12723] (Fathallah et al. 1993). *DNA sequencing:* The DNA for sequencing was isolated by alkaline lysis according to Sambrook et al. (1989). DsDNA was sequenced by the dideoxy chain termination method (Sanger et al. 1977).

Northern Blot

Total sponge RNA was isolated as described before and subjected to denaturing agarose gel electrophoresis (Pfeifer et al. 1993). Hybridization was performed with the *Eco* RI - *Bam* HI digoxygenin-labelled *G. cydonium* hsp70 cDNA probe under high stringency. The detection of the digoxygenin-labelled probe was performed using CSPD as substrate for alkaline phosphatase (Beck and Koestner 1990).

Western blot

Gel electrophoresis of the extracts was performed in 15% or 10% polyacrylamide gels containing 0.1% $NaDodSO_4$ (SDS-PAGE) according to Laemmli (1970). Total cellular extracts of the sponge were obtained as follows: Frozen sponge was homogenized in liquid nitrogen and transferred to phosphate buffered saline, supplemented with 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation (10,000 x g; 4°C; 30 min) the supernatant was subjected to gel electrophoresis. Semi-dry electrotransfer was performed according to Kyhse-Andersen (1984) onto PVDF-Immobilon P. Filters were processed (Towbin et al. 1979) and incubated with monoclonal anti-hsp70 antibodies after blocking the membranes with 3% bovine serum albumin. The immune complexes were visualized by incubation with anti-mouse IgG (peroxidase conjugated), followed by staining with 4-chloro-1-naphthol.

Results and Discussion

Cloning of gene and deduced aa sequence for hsp70 from *G. cydonium*

We have used the human cDNA to identify and isolate the corresponding cDNA clone from the marine sponge *G. cydonium*. Seven independent clones, each resulting in the same sequence [also at the 5'termini] were analyzed. All clones contained the 2.3-kb long cDNA insert. The deduced aa of the open reading frame for the sponge hsp70 is shown in Fig 1. The 655 aa long sequence has a deduced M_r is 72,593.

The aa sequence of *G. cydonium* hsp70, GCHSP70, was aligned (Fig. 1) with hsp70 sequences of the group of cytoplasmic- [cow (BOV-C)], endoplasmic reticulum- [human (Homo-e)], mitochondrial- [*Trypanosoma cruzi* (Tryp-M)], chloroplast- [*Porphyra umbilicalis* (Por-p)] and eubacterial hsp70s [*Escherichia coli* (Eco)] as summarized by Boorstein et al. 1994. Two groups of hsp70s exisit with respect to their inducibility, (i) inducible proteins (ii) and constitutively expressed. The latter ones can be located in a variety of cellular compartments, the cytoplasm, the endoplasmic reticulum in mitochondria, and in chloroplast. The sponge hsp70 shows highest homology to the cytoplasmic hsp70 from bovine. Two consensus sequences have been described for hsp70 (Bairoch 1991); one is [IV]-D-L-G-T-T-x-S (found in the sponge sequence at aa 10 to 17) and a second one is D-[LF]-G(3)-T-F-D (which is present only with four aa ; aa 216 to 219).

Northern blot analysis

Sponge hsp70 mRNA was identified on Northern blots using GCHSP70 as a probe. As shown in Fig. 2 A, its size is 2.3 kb.

Determination of induction of sponge hsp70 by Western blotting

The existence of hsp70 in *G. cydonium* tissue was detected by Western blotting. In samples from untreated tissue only a faint band of 70 kD could be visualized using anti-hsp70 antibodies (Fig. 2B; lane a). Hsp70 appeared in tissue which had been heat treated (Fig. 2B; lane b).

Conclusion

The stress proteins, the "classic" heat shock proteins, have a high potential as biomarkers for general stress (Sanders 1990). In earlier reports and in the present study it is shown that this heat shock response is a fundamental aspect of cellular physiology also in freswater- and marine sponges, in which exposure to temperature stress (Müller et al. 1995a and herein) or xenobiotics (Batel et al. 1993) result in a dramatic reaction, induction of hsps [especially hsp70] and a suppression of other cellular proteins.

With the isolation of the cDNA, coding for hsp70 in the marine sponge *G. cydonium,* a tool is now available to analyze for the first time the reaction of a sponge to stressors on molecular level.

Acknowledgements

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Fig. 1. Alignment of the aa sequence for hsp70, deduced from the cDNA isolated from the marine sponge *G. cydonium* [GCHSP70]. Aligment was performed with hsp70s from cow (BOV-C), human (Homo-e), *Trypanosoma cruzi* (Tryp-M), chloroplast from *Porphyra umbilicalis* (Por-p) and *Escherichia coli* (Eco). Identitys are indicated with full circles [.] homologies with asterisks [•]. The signatures, characteristic for hsp70, are underlines

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GCHSP70   MAKKAPAVGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFT-DSERLIGDAAKNQVAMNPTNTVFDAKRFIGRRSNDPVVSSDKKH   90
BOV-c      MSKGPAVGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFT-DTERLIGDAAKNQVAMNPTNTVFDAKRLIGRRFDDAVVQSDMKH
Homo-e    KKEDVGVVGIDLGTTYSCVGVFKNGRVEIIANDQGNRITPSYVAFTPEGERLIGDAAKNQLTSNPENTVFDAKRLIGRTWNDPSVQQDIKF
Tryp-M    SKVTGDVIGIDLGTTYSCVAVMEGDKPRVLENTEGFRATPSVVAF-KGQEKLVGLAAKRQAVTNPQSTFFAVKRLIGRRFEDSNIQHDIKN
Por-p        MGKVVGIDLGTTNSVIAVMEGGKPTVIPNAEGFRTTASVVAYTKSGDKLVGQIA-RQAVINPENTFYSVKRFIGRKQN--EISQEIRQ
Eco 1         GKIIGIDLGTTNSCVAIMDGTTPRVLENAEGDRTTPSIIAYTQDGETLVGQPAKRQAVTNPQNTLFAIKRLIGRRFQDEEVQRDVSI
              •  •********•*•••••• •••• •• * •* *•*•* •*••   • *•* •*••*    ** •* •  **•****•  •    • • •

GCHSP70   WSFEVI-DEAGRPRRVEYKGEKKSSFGEEFVHCAKKKSFFLEEISSMVLTKMKETAEAYLGKTRRITDAVVTVPAYFNDSQRQATKDAGII 180
BOV-c     WPFMVVNDAGRPKVQVEYKGETKSFYPEEV-------------SSMVLTKMKEIAEAYLGKT--VTNAVVTVPAYFNDSQRQATKDAGTI
Hom-e     LPFKVVEKKTKPYIQVDIGGGQTKTFAPEEI------------SAMVLTKMKETAEAYLGK--KVTHAVVTVPAYFNDAQQATKDAGTIA
Tryp-M    VPYKIGRSSNGDAWV--QD-ANGKQYSPSQV------------GAFVLEKMKETAENFLGR--KVSNAVVTCPAYFNGPQRQATKDAGTI
Por-p     SYNVKTSGSSIKIECP---ALNKDFAPEEI-------------SAQVLRKLVEDASTYLGE--TVTQAVITVPAYFNDSQRQATKDAGKI
Eco-1     MPFKIIAADNGDAWV--E--VKGQKMAPPQI------------SAEVLKKMKKTAEDYLGEP--VTEAVITVPAYFNDAQRQATKDAGRI
           ••                          •             *• ** *••• ** •**      •* ***•******** *••••**•• •

GCHSP70   SGLNILRIINEPTAAAIAYGLDKKHDSSEQNILIFDLGG-----------------FEVKSTAGDTHLGGEDFDNRMVNHFISEFKRKFK 252
BOV-c     AGLNVLRIINEPTAAAIAYGLDKKVGA-ERNVLIFDLGGGTFDVSILTIEDG----IFEVKSTAGDTHLGGEDFDNRMVNHFIAEFKRKHK
Homo-e    GLNVMRII-NEPTAAAIRAYGLDKRE-GEKNILVFDLGGGTFDVSLLTIDNG----VFEVVATNGDTHLGGEDFDQRVMEHFIKLYKKKTG
Tryp-M    AGLNVIRVVNGPTAAALAYGLDK-T-KDSMIAVY-DLGGGTFDISVLEIAGG----VFEVKATNGDTHLGGEDFDLCLSDYILTEFKKSTG
Por-p     AGLDVLRIINEPTAASTLSYGLDKQN--NETILVFDLGGGTFDVSILEVGDG----VFEVLSTSGDTHLGGDDFDQQIVEWLIKDFKQSEG
Eco-1     AGLEVKRIINEPTAAALAYGLDKGTGNRTIAVY--DLGGGTFDISIIEIDEVDGEKTFEVLATNGDTHLGGEDFDSRLINYLVEEFKKDQG
          •*••• •••*******• ••• ••            ••*****•••• •       •     *** •* ***********•          ••*   •

GCHSP70   KDMSGNKRAVRRLRTACERAKRTLSSITEASIEIDSLFEGI----DYYTKITRARFEELCGDLFRGTLEPVEKALRDSKFDKGQIHEIVLV 338
BOV-c     KDISENKRAVRRLRTACERAKRTLSSSTQASIEIDSLYEGI----DFYTSITRARFEELNADLFRGTLDPVEKALRDAKLDKSQIHDIVLV
Homo-e    KDVRKDNRAVQKLRREVEKAK-ALSSQHQARIEIESFYEGE----DFSETLTRAKFEELNMDLFRSTMKPVQKVLEDSDLKKSDIDEIVLV
Tryp-M    IDLSNERMALQRIREAAEKAKCELSTTMETEVNLPFITANQDGAQHVQMTVSRSKFESLAEKLVQRSLGPCKQCIKDAAVDLKEISEVVLV
Por-p     IDLGKDRQALQRLTEASEKAKIELSNLTQTEINLPFITATQDGPKHLEKTVTRAKFEELCSRLIDKCSIPVNNALKDAKLEASSIDEVVLV
Eco-1     IDLRNDPLAMQRLKEAAEKAKIELSSAQQTDVNLPYITADATGPKHMNIKVTRAKLESLVEDLVNRSIEPLKVALQDAGLSVSDIDDVILV
          •*       *  ••• • •*** ***  •  •••    •     •  •     •*•••••*    *        *•   •• *• •   • *••••*•*

GCHSP70   GGSTRIPRIQKLLQDFFNGKTLNKSINPDEAVAYGAAIQADILTGDTSEEVQDLLLLDVTPLSLGIETAGGVM--LIKRNSTIPKKETET- 425
BOV-c     GGSTRIPKIQKLLQDFFNGKELNKSINPDEAVAYGAAVQAAILSGDKSENVQDLLLLDVTPLSLGIETAGGVMTVLIKRNTTIPTKQTQT-
Homo-e    GGSTRIPKIQQLVKEFFNGKEPSRGINPDEAVAYGAAVQAGVLSGD--QDTGDLVLLHVCPLTLGIETVGGVMTKLIPSNTVVPTKNSQI-
Tryp-M    GGMTRMPKVIEAVKQFF-GRDPFRGVNPDEAVALGGATLGGVLRRD----VKGLVLLDVTPLSLGVETLGGVFTRMIPKNTTIPTKKSQTF
Por-p     GGSTRIPAIQQMVKRLI-GKDPNQSVNPDEVVAIGAAVQAGVLAGE----VKDILLLDVTPLSLGVETLGGVMTKIIPRNTTIPTKKSEV-
Eco-1     GGQTRMPMVQKKVAEFF-GKEPRKDVNPDEAVAIGAAVQGGVLTGDVK---DVLLLDVTPLSLGIETMGGVMTTLIAKNTTIPTKHSQV-
          **•***•* ••       •••*• •   •******** ***•••••* ••    • •••**•*•**•*•*•** *****•• •*• *••••*• ••

GCHSP70   FTTYSDNQPGVLIQVYEGERAMTKDNNLLGKFELTGIPPAPREVPQIQVTFDIDRNGILNVSAADKSTGKENKITITNDKGRLSAEEIDRM 515
BOV-c     FTTYSDNQPGVLIQVYEGERAMTKDNNLLGKFELTGIPPAPRGVPQIEVTFDIDANGILNVSAVDKSTGKENKITITNDKGRLSKEDIERM
Homo-e    FSTASDNQPTVTIKVYEGERPLTKDNHLLGTFDLTGIPPAPRGVPQIEVTFEIDVNGILRVTAEDKGTGNKNKITITNDQNRLTPEEIERM
Tryp-M    FSTAAFNQTQVGIKVFQGEREMAADNQMMGQFDLVGIPPAPRGVPQIEVTFDIEPNGICHVTAKDKATGKTQNITITASGG-LSKEQIERM
Por-p     FSTAVDNQPNVEIQVLQGERELTKDNKSLGTFRLDGIMPAPRGVPQIEVTFDIDANGILSVKAKEKATGKEQSITISGAST-LPKDDVERM
Eco-1     FSTAEDNQSAVTIHVLQGERKRAADNKSLGQFNLDGINPAPRGMPQIEVTFDIDADGILHVSAKDKNSGKEQKITIKASS-GLNEDEIQKM
          *•*• ***• * * * •***  ••**  •* * *•**•••**•••***•**•• •**• * * •* •*••• ****      *  •••••*

GCHSP70   VREAEQYKAADDAQRERVSAKN--QSYAFQMKSTFEEDKVKEKVPEEDREKVISKCKEVIDWLDKNQSAEKEEFEHQQKELEGICTPIVTK 603
BOV-c     VQEAEKYKAEDEKQRDKVSSKNSLKSYAFNMKATVEDEKLQGKINDEDKQKILDKCNEIINWLDKNQTAEKEEFEHQQKELEKVCNPIITK
Homo-e    VNDAEKFAEEDKKLKERIDTRNELESYAYSLKNQIGDKEKLGGKLSSEDKETMEKAVEEKIEWLESHQDADIEDFKAKKKELEEIVQPIIS
Tryp-M    IRDSESHAESDRLKRELVEVRNNAETQANTAERQLTEWKYVSDAEKENVRTLLRACRKSMENPNVTKDELSAATDKLQKAVMECGRTEYQQ
Por-p     VKEAEENFDVDQKRRKNIDIRNQAESLCYQSEKQVKE---FEDKIDEELKNRITNLISELRSNLEKEELDSIEANSEKLQNALMEIGKNAT
Eco-1     VRDAEANAEADRKFEELVQTRNQGDHLLHSTRKQVEE---AGDKLPADDKTAIESALTALETALKGEDKAAIEAKMQELAQVSQKLMEIAQ
          • •••     * • • • •*   • •     • •                    •           •

GCHSP70   LYQAGGAPGGGMPGGMPGGMPGGMPGGFPGGAGPTSGGSTGGGSGPTIEEVD                                       655
BOV-c     LYQSAGGMPGGMPGGMPGGFPGGGAPPSGGASSGPTIEEVD
Homo-e    KLYGSAGPPPTGEEDTAEKDEL
Tryp-M    AAAGNSSSSSGNTDSSQGEQQQQGDQQKQ
Por-p     SAEKDTQNASNDDTVIDTDFSEAK
Eco-1     QQHAQQQTAGADASANNAKDDDVVDAEFEEVKDKK
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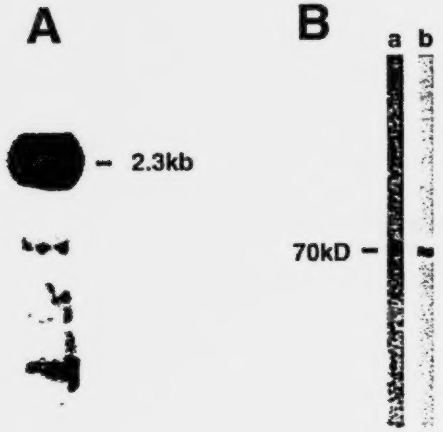
Fig. 2. A. Identification of *G. cydonium* GCHSP70 mRNA by Northern blotting. RNA was isolated and 10 µg was resolved by electrophoresis in agraose. The RNA was transferred to Nylon membrane and hybridized with labelled cµg was resolved by electrophoresis in agraose. The RNA was transferred to Nylon membrane and hybridized with labelled GCHSP70 clone. **B.** Identification of hsp70 in extracts (15 µg each) from *G. cydonium*, which remained untreated (lane a) or had been treated by temperature stress (lane b). The samples were size-separated by SDS gel electrophoresis, transferred to nitrocellulose sheets and incubated with anti-hsp70 antibodies. The apparent molecular mass is given (in kD).



SMALL-SCALE *in vitro* GENOTOXICITY TESTS FOR BACTERIA AND INVERTEBRATES

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Monitoring of the effects of environmental toxic and genotoxic substances has gained increased importance in recent times. Assessment of environmental samples for the presence of genotoxins (carcinogens and mutagens) have therefore become valuable approaches for evaluating genotoxic potentials of environmental pollutants.

Overlapping with this level of functional component, effects at the organismic level can be perceived when organisms are exposed longer to these environmental pollutants. Effects at this level would eventually result in contaminant genotoxic effects at macromolecular levels such as DNA-damage, mutagenesis, etc. Since environmental genotoxicity assessment approaches provide information on such effects they would be the potential environmental monitoring tools. Table 1 gives information on the consequences of DNA damage at the organisational levels of biological systems.

Table 1. Consequences of DNA damage of different organisational levels of biological systems.

Level of biological organisation	Effects
DNA	-mutations
Cell	-cell death -disordered proliferation and differentiation -neoplastic transformation
Tissue / Organ	-functional defects -malformations -tumors
Organism	-reduced viability -reduced fertility
Population	-reduction of population size -extinction
Ecosystem	-reduction of species diversity

Extended exposure of organisms to environmental genotoxins would result in several physiological disorders such as reproductive impairments and other related abnormalities. Therefore, the response measurements to reproductive toxicity is essential for assessing the effects of anthropogenic sources.

Several biomonitoring approaches currently exist. For the organisational level of the bacteria there is the umuC-assay and for the invertebrates (mussels) the DNA-unwinding assay as monitoring tools available. Both test systems are adapted to micro scale assays (microplate technique). And even the development of a genotoxicity sensor using the concept of the umu test system is in progress.

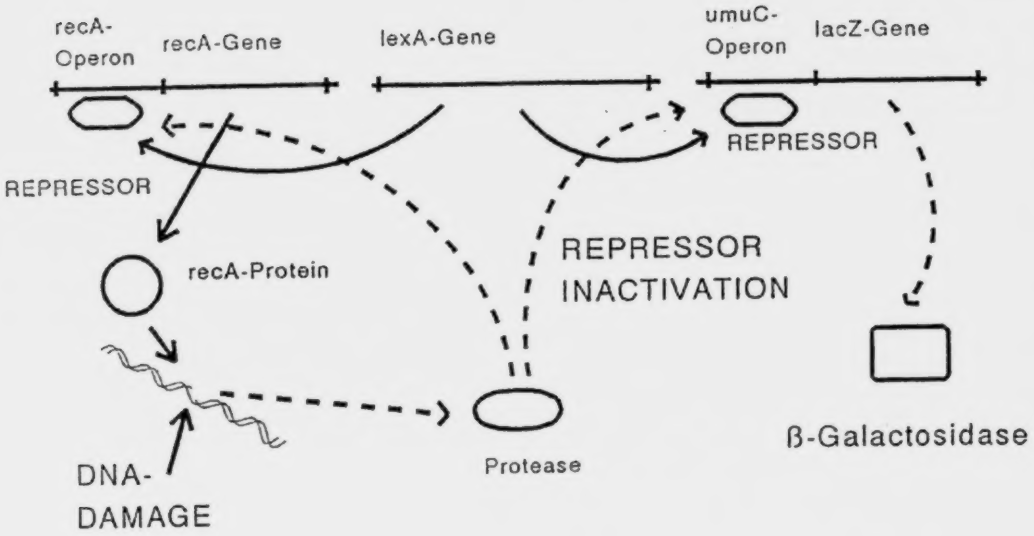

Fig. 1: Principle of the umuC-assay

The German Institute for Standardisation (DIN) has recently standardized the umuC-assay as an official protocol for monitoring environmental genotoxicity (DIN 38415, part 3). For this test system the genetically engineered bacterium *Salmonella typhimurium* TA1535/pSK1002 serves as test organism. The assay will be standardized under ISO (International Standardization Organisation) in the working group 9 "Genotoxicity". It is already a committee draft (CD): ISO/TC 147/SC5/WG9-N8. The bacteria are exposed under controlled conditions to different concentrations of the samples to be tested. The test is based on the capability of genotoxic agents to induce the umuC operon in the Salmonella strain in response to genotoxic lesions in the DNA. Due to its capability to respond to different types of genotoxic lesions, only one single strain is necessary to detect different kinds of genotoxic substances. The induction of the umuC operon is thus a measure for the genotoxic potential of the material tested. Since the umuC operon is fused with the lacZ-gene for the β -galactosidase, the induction of the umuC operon can be easily assessed by the determination of the β-galactosidase activity.

The DNA-Unwinding assay is known to be a promising tool with eukaryotic cells for detecting DNA damage due to environmental genotoxins in aquatic animals. Extensive work is currently underway to develop a basis for uniform application of this bioassay for environmental samples. Recent work on the application of this protocol has clearly indicated its usefulness as genotoxic monitoring tool. DNA damaging activity in mussel hemocytes (*Mytilus edulis*) was observed after 96 h static exposure to genotoxic chemicals. Recent investigations indicate that the DNA damage in *M. edulis* is increased in the stressed area in the harbour compared to an unpolluted reference station.

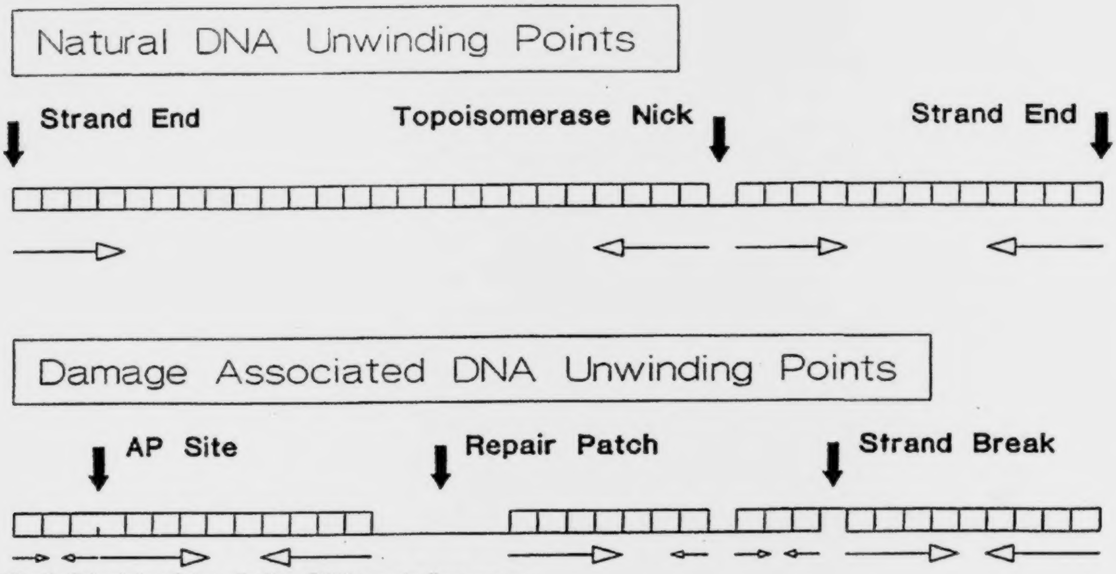

Fig.2: Principle of the alkaline DNA unwinding assay

In the future environmental monitoring approaches should include measurements of DNA damage in mussels and umu-assay in other environmental samples. Finally, environmental mapping of hot spots for the genotoxic potential should be made mandatory for assessing and maintaining ecosystem health.

Conclusions

The umu-assay is a helpful tool for the management of sewage farms due to the genotoxic potential of hot spots and for highly polluted surface waters as well. The DNA-Unwinding assay is useful for assessing stressed areas for coastal zone and river management. The umu-assay is a prokaryotic test system while the DNA-Unwinding assay is a eukaryotic one. The umu-assay is available as a Committee Draft by ISO. Both test systems are adapted to micro-scale assays (microplate technique). The development of a genotoxicity sensor is in progress.

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MARINE AND ESTUARINE POREWATER TOXICITY TESTING

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The porewater toxicity test approach has been used extensively in recent years to assess the quality of marine and estuarine sediments. Numerous methods (e.g., centrifugation, pneumatic, vacuum, passive) have been used to extract interstitial (pore) water from sediments. The results from these studies suggest that any of these methods yield samples exhibiting similar toxicity, if care is taken in the selection of materials that contact the sample. The effects of various sediment storage effects and manipulations have also been investigated. If care is taken to remove suspended particulates (by performing a second centrifugation after the initial extraction), freezing and thawing do not affect the toxicity of porewater samples.

One of the primary advantages of the porewater approach is that sensitive life-stages of sensitive species are amenable to testing unlike the more commonly employed whole-sediment tests. Tests that have been used successfully with pore water include [1] a polychaete life-cycle test, [2] survival and growth test with copepod nauplii, [3] fertilization, cytogenetic, and embryological development assays with sea urchins, [4] survival, hatching success, and larval development test with fish, and [5] algal zoospores germination and germling growth test. Recent studies have demonstrated comparable sensitivity among the sea urchin, polychaete, and copepod tests but differences among the tests commonly occur due to the contaminant-specific nature of the different mechanisms of toxicity measured by the various tests. This result supports the contention that a suite of sensitive tests is preferable to a single species or end point.

The standard 10-d amphipod survival test has been tested in side-by-side comparisons with the sea urchin fertilization and embryological development assays in numerous recent sediment quality assessment surveys (totalling more than 600 samples) in bays and estuaries along the Atlantic and Gulf coasts of the US. The results of these comparisons have demonstrated that the sea urchin porewater tests are always considerably more sensitive (by at least an order of magnitude on average) than the amphipod whole-sediment test which is the most sensitive test used for regulatory testing of dredged material in the US. This difference in sensitivity is not entirely unexpected as the amphipod test measures acute toxicity with an adult benthic crustacean whereas the sea urchin fertilization and embryological development tests with pore water provide measures of chronic sublethal impacts on sensitive life-stages of a sensitive organism. Numerous recent sediment surveys have shown excellent agreement between toxicity predicted using sediment quality assessment guidelines and toxicity observed with porewater toxicity tests. A high degree of association has also been observed between sediment contaminant concentrations and sea urchin porewater toxicity tests. We believe that the use of more sensitive tests and test species is needed to provide the information necessary to make informed decisions about the disposal of dredged material in oceanic and near coastal environments.

THE USE OF LARVAE AND SMALL SPECIES OF POLYCHAETES IN MARINE TOXICOLOGICAL TESTING

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Polychaetous annelids were used initially as toxicological test animals 35 yr ago, but only extensively in the past 15 yr. Polychaetes are important component of the marine benthic environment where they constitute 35-50% of both the total macroscopic species and specimen population. Tests with polychaetes have been conducted which measured the effects of toxicants in the water, sediment and interstitial water. The majority of the tests have utilized adult animals. Tests have been conducted with over 40 species from 19 families.

Polychaete embryos and juvenile stages have been employed to a limited extent. Complete life cycle tests have been conducted in which the experiment is initiated with trochophore larvae (i.e. *Capitella capitata*) or juveniles (i.e. *Neanthes arenaceodentata*). Very little difference has been noted in the response to a toxicant between these young stages and adults on the basis of limited studies. The effect of a toxicant upon gametogenesis and reproduction appears to be the most sensitive period in the life cycle of polychaetes. Polychaetes with a short life cycle also are those which are small in size (<5 mm in length); the most commonly used species are *Dinophilus gyrociliatus*, *Ctenodrilus serratus*, and species within the genus *Ophryotrocha*. Selection of embryos or young stages for testing has been dependent upon those species which are routinely cultured in the laboratory. Species which are routinely cultured include *C. capitata*, *C. serratus*, *N. arenaceodentata*, *Nereis diversicolor*, *Ophryotrocha diadema*, and *O. labronica*. The primary criterium for selection has been convenience; specimens are always available and the investigator is independent upon the whims of environmental conditions. It must be realized that development of a new laboratory test animals, such as occurring in Canada today, requires time.

MICROSCALE AQUATIC TESTING WITH EARLY LIFE STAGES OF KILLIFISH

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The common killifish, or mummichog (*Fundulus heteroclitus*), a hardy Cyprinodont resident of western Atlantic coast estuaries, is frequently used in aquatic toxicity testing. Because early life stages are particularly sensitive, and gametes of this species are available during a long breeding season, embryos and larvae have been used by many investigators. Not only mortality but also sublethal endpoints have been reported in response to pesticides, oil components, and metals. Congenital malformations of the head, cardiovascular and skeletal systems, as well as developmental arrest, physiological deficiencies and hatching delay are possible responses. Another characteristic of the mummichog is a restricted home range, which allows populations to be compared. Striking differences in embryonic responses to contaminants between populations from polluted vs. pristine sites have been reported. As larvae, the protective chorion is no longer present, and behavior, a particularly sensitive parameter, can be studied, as well as the more traditional toxicological responses. Thus, the early life stages of the mummichog present many advantages to the aquatic toxicologist because of the variety of endpoints, ready availability, and economy of scale.

GENOTOXICITY IN FISH EGGS/EMBRYOS

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Genotoxicity, i.e. the potential to alter harmfully the genetic information carrier DNA in germ line or somatic, cells, has traditionally been considered as a key event in mutagenesis and carcinogenesis. However, in the recent past it was also discussed as possible cause for a variety of other impairments, since DNA is the primary information matrix in living organisms and is thus template for development and stability of all essential biological structures and functions (Herbert and Hansen 1992, Kurelec 1993). Among other consequences of DNA damage, disturbation of embryonic development - where by far most of the genetic information is realized (Galau et al. 1976)- has to be considered as a major problem, since it may interfere with fertility, which is supposed to have major relevance to population biology.

DNA damage was investigated in fish embryos caught in the North Sea, where it has now been documented since many years, that embryonic malformations occur at high incidences (Cameron et al. 1992). Data were collected during two sea cruises in 1993 and 1995. During the latter, effects on different developmental stages were also investigated. The applied methodology was alkaline DNA unwinding/HAP 'batch' elution.

Alkaline DNA unwinding/HAP 'batch' elution provides a technical approach to investigate DNA damage in fish eggs/embryos as well as in adult fish and aquatic invertebrates (Herbert 1987, Herbert and Zahn 1989, 1990, Herbert 1990, Hansen et al. 1995, Herbert et al. 1995a, b). A wide spectrum of different DNA lesions are detected and quantified by the sum paramter of alkali sensitivity. The method has been applied to studies with dab (*Limanda limanda*) embryos from the North Sea. Results indicate a distinct spatial pattern of genotoxic impact with widely occurring severe maxima. The pattern is similar in early and late developmental stages. Genotoxic effects in the embryos exceed similar effects in adult dab liver by an order of magnitude.

Embryos were also exposed experimentally to artificial sunlight to investigate possible effects of UV light on fish embryo DNA integrity. Exposed to spectrum conditions of Orlando, Florida/USA, with simulated 50% atmospheric ozone depletion, no genotoxic effects in fish embryos were detectable up to doses with acute toxic effects. This supports the hypothesis, that the observed DNA damage is associated mainly with pollution impact.

The severe genotoxic effects in fish embryos related to environmental exposure are discussed in context with similar findings of other authors. Possible consequences on the population and community (ecosystem) level require further delineation.

Conclusions

Monitoring the effects of genotoxic substances has gained increased importance. DNA damage interferes with fertility and has relevance to population biology. The DNA-Unwinding method has been applied to studies with fish embryos from the North Sea. Results indicate a distinct spatial pattern of genotoxic activity with widely occurring severe peaks of impact. The pattern is similar in early and late developmental stages of the fish larvae and adult fish as well. Possible effects of UV light on fish embryo DNA integrity were investigated, but the observed DNA damage was associated mainly with the pollution impact.

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COMPARISON OF MICRO-SCALE AND STANDARD ACUTE TOXICITY TESTS IN ASSESSING THE TOXICITY OF EFFLUENTS FROM METAL MINING SITES ACROSS CANADA

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In 1992, Environment Canada initiated a public review process aimed at assessing current Federal regulations controlling mining effluent discharges in Canada. In response to the major recommendation from the public review, the Aquatic Effects Technology Evaluation (AETE) program was established to review techniques for monitoring the impacts of mine effluents on the aquatic environment. AETE is a cooperative effort of the Canadian mining industry, federal government and several provincial governments. The program includes three areas: biological monitoring in receiving waters, water and sediment monitoring, and toxicity testing. The objective is to identify affordable and effective tools for determining and characterizing the impacts of mine effluents on receiving waters. The program is undertaken from a technical and economic perspective and purposely avoids the regulatory aspect of effluent monitoring and control.

This $3.4 million program is funded by CANMET and the Mining Association of Canada (MAC), and directed by a consortium of federal, provincial and industrial partners. A series of reports on appropriate, cost-effective methods of determining the biological and non-biological impacts of mine effluents on Canada's lakes, rivers and streams will be generated during the program which began in April 1994 and will conclude in March 1998.

The first component of the toxicity testing sub-program, acute toxicity, has two principal goals: [1] to evaluate practical cost-effective test alternatives to the standard acute toxicity tests, the 96 h rainbow trout (EPS 1/RM/13) and the 48 h *Daphnia magna* (EPS 1/RM/14) tests; [2] to correlate toxicity test results to effluent chemistry. Five rapid, micro-scale acute toxicity tests were compared to the current standard regulatory tests, using mine effluents representing the major mine types across Canada. The micro-scale tests assessed during this project included: Microtox™; Rotoxkit F™; Thamnotoxkit F™; Toxichromotest™; *D. magna* IQ™.

The results of the five alternative tests were evaluated against results of the two standard regulatory tests, with respect to cost, sensitivity, speed, accuracy, applicability and reproducibility. This testing was umdertaken to determine which, if any, micro-scale tests can provide effluent toxicity information comparable to that from standard tests, which are slow and, especially in the case of trout test, expensive.

Treated final effluent samples were collected by mine operators from 21 mine sites (5 Au, 4 Cu/Zn, 4 Ni/Cu, 3 Pb/Zn, 2 U, 1 Zn, 1 Sn, 1 Bitumen) over a period of 5 months (total of 64 samples). The samples were shipped by courier to the laboratories involved in the program, as follows: [1] BAR Environmental in Guelph, Ontario (for rainbow trout, *D. magna*, and *D. magna* IQ); [2] BEAK Consultants in Dorval, Québec (for Microtox, Rotoxkit F, Thamnotoxkit F and Toxichromotest); [3] Seprotech Laboratories in Ottawa, Ontario (for the chemical analyses). For QA purposes, approximately 12% of the effluent samples were split and sent to 3 volunteer government laboratories for toxicity testing (Environment Canada and Ontario Ministry of Environment and Energy) and chemical analyses (CANMET).

The results were collated and are being statistically analyzed by Pollutech Enviroquatics. Pollutech will report on the feasibility of the alternatives in terms of cost, sensitivity, speed (turn around time), accuracy, applicability, and reproducibility. A final report will be available in December 1995.

Since the data interpretation is not finished (statistical analyses, correlation with effluent chemistry), final conclusions cannot be presented here. The raw data reveals some information, however, only 12 of the 21 mine sites sampled had effluents which were acutely lethal to rainbow trout or *D. magna*. Effluents from 18 of the 21 sites had effluents toxic to one or more of the alternative micro-scale tests. Of the 64 effluent samples tested, 19 were toxic to one or more of the alternative micro-scale tests but non-acutely lethal to rainbow trout or *D. magna*; 2 were acutely lethal to rainbow trout or *D. magna* but non toxic to all alternative micro-scale tests; 30 were acutely lethal to rainbow trout or *D. magna*; 50 were acutely toxic to one of the tests conducted; and 13 did not demonstrate any toxicity. Data for each type of mine effluent are presented in Tables 1 to 5, following.

The data suggests that for effluent samples that produced a toxic response to the standard tests, the alternative test which provided the best correlation response was the *D. magna* IQ test. In many cases, the IQ test was more sensitive than the *D. magna* or the rainbow trout lethality tests. The next most responsive test was the Thamnotoxkit F which seemed to correlate well with the *D. magna* lethality test. Microtox provided some positive response corresponding to the standard tests. The least responsive tests were the Toxichromotest and the Rotoxkit F. It should be emphasized that these conclusions are preliminary, pending completion of the statistical review.

There has been recent interest in reviewing current regulations in mitigating mining effluent impacts on ecosystems. As part of this initiative, the Aquatic Effects Technology Evaluation (AETE) program was established to review technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, federal government and several provincial governments. The first project of the AETE program is a comparison of five rapid, cost effective acute toxicity tests alternatives to the standard regulatory tests: the 96 hr rainbow trout and 48 h *Daphnia magna* acute lethality tests, using mine effluents representing the major mine types across Canada. The micro-scale tests assessed during this project include Microtox™, Rotoxkit™, Thamnotoxkit™, Toxichromotest™ and *D. magna* IQ™. The results of each alternative test have been compared against results from the standard acute toxicity tests in terms of cost, sensitivity, speed, accuracy, applicability and reproducibility. A summary of project results for each test will be presented.

Table 1. Primary lab results for gold mine effluents (expressed as % vol/vol).

Site Number	Sampling Period	Rainbow trout (96 hr LC50)	*Daphnia magna* (48 hr LC50)	*Daphnia magna* IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24 hr LC50)	Thamno-toxkit (24 hr LC50)	Toxichro motest (90 min IC50)
Au #1	February	N.L.	N.L.	33.7	>4.9	>100	N.L.	>50
	March	N.L.	N.L.	N.E.	>49.5	N.L.	>100	>50
	May	>100	63.0	6.5	78.2	8.7	45.3	>50
	June	77.1	44.5	2.1	>99	2.0	20.6	6.3
Au #2	February	>100	N.L.	>100	>49.5	>100	>100	>50
	March	N.L.	N.L.	>100	>90	>100	>100	>50
Au #3	February	N.L.	N.L.	>100	>90	>100	>100	>50
	March	N.L.	N.L.	>100	>90	N.L.	>100	>50
Au #4	May	43.5	13.3	0.2	54.7	6.4	17.2	6.3
	June	43.5	15.0	0.5	60.5	5.5	18.3	11.0
Au #5	May	N.L.	>100	15.9	>99	>100	>100	>50

N.L. = Non-lethal N.E. = No effect

Table 2. Primary lab results for Cu/Zn mine effluents (expressed as % vol/vol).

Site Number	Sampling Period	Rainbow trout (96 hr LC50)	*Daphnia magna* (48 hr LC50)	*Daphnia magna* IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24 hr LC50)	Thamno-toxkit (24 hr LC50)	Toxichro-motest (90 min IC50)
Cu/Zn #1	February	89.1	73.0	78.5	>49.5	>100	>100	>50
	March	N.L.	>100	>100	>49.5	N.L.	82.9	>50
	May	70.7	>100	>100	49.6	>100	59.1	>50
	June	89.1	>100	37.5	>99	N.T.	58.4	>50
Cu/Zn #2	February	N.L.	>100	>100	>90	>100	>100	>50
	March	N.L.	70.7	10.8	>90	>100	>100	>50
	June	70.7	70.7	70.7	>99	N.T.	>100	>50
Cu/Zn #3	February	N.L.	N.L.	89.9	>90	>100	42.8	23.7
	March	N.L.	>100	N.E.	>90	>100	49.8	>50
	May	N.L.	>100	70.7	>99	>100	35.0	9.0
	June	>100	N.L.	>100	>99	70.7	70.7	5.8
Cu/Zn #4	March	53.6	75.0	9.9	>49.5	>100	41.0	>50
	May	43.5	>100	18.3	>99	75.3	60.2	>50
	June	53.6	N.L.	72.4	>99	N.T.	36.0	>50

N.L. = Non-lethal N.E. = No effect N.T. = Not tested

Table 3. Primary lab results for Ni/Cu mine effluents (expressed as % vol/vol).

Site Number	Sampling Period	Rainbow trout (96 hr LC50)	*Daphnia magna* (48 hr LC50)	*Daphnia magna* IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24 hr LC50)	Thamnotoxkit (24 hr LC50)	Toxichromotest (90 min IC50)
Ni/Cu #1	February	70.7	N.L.	72.3	>49.5	>100	>100	>50
	March	70.7	N.L.	31.3	>49.5	N.L.	>100	>50
	May	70.7	70.7	8.0	>99	N.L.	N.L.	>50
	June	70.7	N.L.	17.2	>99	N.T.	>100	>50
Ni/Cu #2	February	82.0	N.L.	>100	>49.5	>100	>100	>50
	March	N.L.	N.L.	40.0	>90	>100	>100	>50
Ni/Cu #3	February	N.L.	N.L.	34.1	>4.9	N.L.	>100	>50
	March	N.L.	N.L.	N.E.	>90	>100	N.L.	>50
	May	N.L.	N.L.	>100	>99	N.L.	N.L.	>50
	June	N.L.	N.L.	>100	>99	N.T.	>100	>50
Ni/Cu #4	May	70.7	70.7	51.7	22.2	70.7	N.L.	>50
	June	>100	73.0	14.0	62.3	62.6	>100	>50

N.L. = Non-lethal N.E. = No effect N.T. = Not tested

Table 4. Primary lab results for Pb/Zn mine effluents (expressed as % vol/vol).

Site Number	Sampling Period	Rainbow trout (96 hr LC50)	*Daphnia magna* (48 hr LC50)	*Daphnia magna* IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24 hr LC50)	Thamnotoxkit (24 hr LC50)	Toxichromotest (90 min IC50)
Pb/Zn #1	February	70.7	>100	29.8	38.2	>100	48.0	>50
	March	70.7	70.7	63.0	>90	N.L.	46.8	>50
	May	N.L.	>100	N.E.	>99	>100	62.4	>50
	June	N.L.	>100	>100	>99	>100	91.4	22.0
Pb/Zn #2	February	N.L.	N.L.	37.2	>4.9	>100	49.3	>50
	March	N.L.	>100	>100	>49.5	N.L.	8.5	>50
	May	>100	70.7	29.8	>99	78.3	42.0	>50
	June	73.5	73.0	>100	N.T.	N.T.	N.T.	N.T.
Pb/Zn #3	February	N.L.	39.7	>100	>90	>100	9.4	>50
	March	N.L.	70.7	61.0	>49.5	>100	11.2	>50
	June	N.L.	70.7	>100	>99	>100	>100	>50

N.L. = Non-lethal N.E. = No effect N.T. = Not tested

Table 5. Primary lab results for U, Zn, Bitumen and Sn mine effluents (expressed as % vol/vol).

Site Number	Sampling Period	Rainbow trout (96 hr LC50)	*Daphnia magna* (48 hr LC50)	*Daphnia magna* IQ (75 min EC50)	Microtox (15 min IC50)	Rotoxkit (24 hr LC50)	Thamnotoxkit (24 hr LC50)	Toxichromotest (90 min IC50)
Uranium #1	February	N.L.	>100	>100	>49.5	>100	>100	>50
	March	N.L.	>100	>100	>90	N.L.	>100	>50
Uranium #2	May	N.L.	N.L.	75.5	>99	>100	N.L.	>50
	June	N.L.	>100	96.7	>99	>100	N.L.	>50
Zn	February	N.L.	N.L.	87.8	>4.9	>100	59.3	>50
	March	N.L.	N.L.	N.E.	>90	>100	56.6	14.8
	May	N.L.	N.L.	N.E.	>99	>100	N.L.	>50
	June	N.L.	N.L.	N.E.	>99	>100	>100	>50
Bitumen	February	35.4	N.L.	34.1	45.6	>100	>100	>50
	March	40.6	N.L.	19.6	41.5	>100	>100	>50
	May	43.5	N.L.	8.4	47.4	>100	N.L.	21.0
	June	35.4	N.L.	22.4	47.6	>100	>100	>50
Sn	February	>100	>100	49.0	>49.5	>100	>100	>50
	March	>100	N.L.	36.1	[illegible]	>100	>100	>50
	May	>100	N.L.	72.2	>99	>100	>100	2.9
	June	>100	N.L.	>100	>99	>100	>100	>50

N.L. = Non-lethal N.E. = No effect

Table 6. Summary of test results.

	Percentage of Positive Responses out of...		
	64 samples collected	23 samples toxic for rainbow trout	17 samples toxic for *Daphnia magna*
Rainbow trout	36%	(100%)	59%
Daphnia magna	27%	43.5%	(100%)
Daphnia magna IQ	59.4%	86.9%	82.3%
Microtox	24%	43.5%	29%
Rotoxkit F	15.7%	21.7%	41.2%
Thamnotoxkit F	39.7%	43.5%	53%
Toxichromotest	15.8%	17.4%	17.6%

APPLICATION AND EVALUATION OF SEDIMENT BIOASSAYS FOR REGULATORY ASSESSMENT OF DREDGED MATERIAL FOR OCEAN DISPOSAL

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A tiered assessment approach for characterization of dredged materials was used by Environment Canada to meet the requirements set out in Part VI of the Canadian Environmental Protection Act. The biological component of this approach comprised of a set of three sediment bioassays and a bioaccumulation test.

Recent studies on sediment assessment using sediment toxicity tests have indicated that the results of these toxicity tests can be affected by factors other than the sediment pollutants. The most well known factors are the particle size composition of the sediment and the "natural contaminants" such as sediment ammonia and sulfide.

This paper will review the effects of sediment particle size, ammonia, and sulfide on the toxicity end-points of bioassays conducted under the Ocean Disposal Program. Discussion will be focused on the interpretation of the bioassay end-points in reference to the bioassay result interpretation protocols developed by Environment Canada for the review of ocean disposal permit applications.

MICROBIOASSAYS AS VALUABLE TOOLS IN WATER MANAGEMENT: ARGENTINEAN/ SOUTH AMERICAN APPLICATION PERSPECTIVES

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An analysis and discussion on the importance of bioassay use in water management is considered, including a brief historical description of ecotoxicological testing. An overview on methods employed by environmental agencies of several countries and their applications, including the advantage of microbioassay testing scale, is also provided.

A discussion on necessary steps in research and development to obtain adequate test methods, including adaptability and applicability according to regional needs prior to the introduction of bioassays in environmental regulations and management in Argentina, is analyzed. The use of existent experience from other countries in methodological considerations, application of methods and results from the application should be taken into account. The importance of autochthonous species use in toxicity testing and significance of their distribution in relation to climatic regions is also considered.

An integrated effort between academic and scientific system, governmental control agencies and productive sector is proposed at all stages of development of regulation laws for environmental control of discharges and assessment of water quality and interpretation guidelines.

APPLICATION OF THE MICROTOX® BIOASSAY TO MONITOR *in situ* BIOREMEDIATION

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Bioremediation strategies have generally received positive responses from the public since an implied goal of bioremediation is that of reducing toxic effects. This technology has been successfully applied on numerous oiled shorelines. However, there is concern that the metabolic by-products from oil degradation, or the components within the bioremediation agents, could be toxic. To address these safety concerns and to verify the effectiveness of bioremediation strategies in mediating toxicity reduction, future operational guidelines will require reliable ecotoxicological test protocols.

Studies have been conducted to evaluate the potential of a modified Microtox® bioassay procedure to monitor the rates of habitat recovery in oiled-beach sediments and the efficacy of bioremediation agents. Based on the results of experimental field studies in Nova Scotia, which included inorganic fertilizers and commercial bioremediation products, this system appears to be a promising monitoring tool for oil spill response. Its operational advantages include sensitivity, availability and ease of use.

USE OF MICRO-SCALE AQUATIC TOXICITY TESTS TO SET "GREEN STANDARDS" FOR GENERAL PURPOSE CLEANERS

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To be authorized to carry an Ecologo^{M} packaging seal, general purpose cleaners (GPC) must comply with bioanalytical guidelines recently imposed by the Canadian Environmental Choice^{M} Program. This is meant to ensure that whole formulation use of such products will not prove toxic to aquatic life once "post-consumer material" has been discarded and where the possibility exists for their entering surface waters. Presently, Ecologo^{M} compliance concentrations for GPC formulations are based, among other criteria, on toxicity results generated with three small-scale tests (*Vibrio fischeri* acute Microtox™ test; *Selenastrum capricornutum* growth inhibition microplate test; *Ceriodaphnia dubia* survival test). The intended manuscript will demonstrate how proposed standards for GPCs were derived and their environmental protection rationale.

AQUATIC TOXICOLOGY OF WATER BIRDS
SESSION CO-CHAIRS: N. BURGESS AND D.V.C. WESELOH

BARNACLE (*Balanus* sp.) CYPRIDS AS A TRANSPORT MEDIUM FOR CONTAMINANTS IN COASTAL BIRDS

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While investigating contaminants in greater scaup (*Aythya marila*) and their winter habitats in Connecticut waters of Long Island Sound, this species and other water birds were observed filter feeding on the surface in spring 1992 upon *Balanus balinoides* cyprids during an unusually early (Feb-Mar) and heavy *Balanus* bloom. Previously unreported, ingestion of cyprids by scaup was confirmed by examination of gizzard contents of 23 lesser scaup (*A. affinis*) and 54 greater scaup. Thirty-two percent of the gizzards of both species from three different locations contained cyprids. These ducks, four other species of waterfowl and two gull species were observed picking at and filtering surface waters at the same time, presumably for the same reason. Plankton net surface tow samples from waters where these birds were feeding verified the abundance of cyprids. Some scaup contained gizzards packed (16 mL) with cyprids (3,200/mL) but no other ingested material. Analyses of cyprids from gizzards for trace elements confirmed the presence of nine elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Zn) and established that this zooplankter can be a key source of metals found in other tissues of these ducks. Metals, especially Cd, Hg, Pb and Se, in plankton tow samples were comparable to those in the cyprids and generally higher than in other scaup foods. Although contamination of greater scaup and lesser scaup may also occur elsewhere in their life cycle via other vectors, it is apparent that ingestion of barnacle cyprids can contribute to the metal load in scaup in Long Island Sound during the critical late winter pre-migration period.

CAUSES OF MORTALITY IN COMMON LOONS IN THE MARITIME PROVINCES

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The health status of common loons (*Gavia immer*) found dead in the field was studied with respect to the occurrence of infectious diseases, degree of intestinal parasitism, and contamination by lead and mercury. Between May 1993 and August 1995, necropsies were performed on 28 birds. Laboratory examination included age determination (immature or adult, based on plumage and gonadal development), assessment of body condition (based on the relative size of the pectoral muscle mass and the abundance of fat reserves), gross and microscopic examination of internal organs, examination of the intestinal content for the presence of helminths (nematodes, cestodes, trematodes), and determination of lead and total mercury in the kidneys (on a wet weight basis).

Six birds were in good body condition and had died acutely from drowning (four) or severe trauma (two). The remaining 22 birds were in poor body condition. Obvious causes of emaciation and death in these birds included lead poisoning (six), severe respiratory aspergillosis (five), and severe oil contamination (five); the cause of emaciation in six birds (four immatures, two adults) could not be determined. All six birds with lead poisoning were adults. The average amount of lead in their kidneys was 91.6 mg/kg (range, 15.5 - 167 mg/kg); remnants of fishing lures were found in the gizzards of four of these birds. Respiratory aspergillosis consisted of severe chronic infection of the air sacs by the fungus *Aspergillus fumigatus*. The five birds severely contaminated by oil had been retrieved from coastal waters.

The results of mercury analysis revealed a significantly higher average amount of this metal in adults in poor body condition (24.0 mg/kg; range 0.5 - 61) as compared to immatures in poor body condition (2.3 mg/kg; range, 0.1 - 2.9), adults in good body condition (3.9 mg/kg; range, 1.9 - 5.6), and immatures in good body condition (1.05 mg/kg; range, 1.0 - 1.1). Mercury is bound to cellular proteins in the body. Gradual emaciation associated with other disease problems (e.g. lead poisoning or respiratory aspergillosis) in the adult group in poor body condition may have resulted in redistribution of mercury bound to muscle proteins into organs such as the kidneys and brain, as the muscle mass was mobilized as an endogenous source of protein.

A pattern comparable to that of mercury was observed with regard to the number of trematodes (mainly *Cryptocotyle* species and echinostomes) in the intestinal content. The average number of these parasites was significantly higher in adults in poor body condition (5,558; range, 0 - 30,080) and in immatures in poor body condition (2,379; range, 0 - 10,340), as compared to adults in good body condition (67; range, 24 - 145) and immatures in good body condition (105; range, 0 - 210). Relative inhibition of the birds' endogenous defense mechanisms caused by poor body condition may have allowed the development of an abnormally large number of these trematodes in their intestinal lumens.

Assuming that a parasitic load of more than 2,500 intestinal trematodes and a body burden of mercury resulting in more than 15 mg/kg in the kidneys have detrimental effects on their avian hosts, particularly when the latter are already debilitated by other diseases, 15 of the 22 loons in poor body condition were affected concurrently by two or more disease processes. In particular, five of the six birds with lead poisoning also had more than 15 mg/kg of mercury in their kidneys, and three of the five birds with severe respiratory aspergillosis concurrently had high mercury levels and a large intestinal parasitic load. The two adult loons for which the cause of emaciation and death could not be determined with certainty also had high mercury levels and a large intestinal parasitic load. These results suggest that studies on the effects of environmental pollutants on wildlife must consider the influence of other disease processes, such as infections and parasitism, which may act synergistically with toxicants to cause morbidity and death.

TEMPORAL AND SPATIAL TRENDS IN CONTAMINANT LEVELS OF HERRING GULL EGGS FROM THE GREAT LAKES, 1974-1993

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The levels of 14 organochlorine contaminants in herring gull (*Larus argentatus*) eggs were measured at up to 13 Great Lakes colonies during the period 1974-1993. Linear regression was applied to all transformed contaminant values at each site to establish statistical patterns. Long-term analysis (1974-1993) consisted of finding an appropriate change point model, comparing its efficacy with a single line model and choosing the most appropriate. Fifty-eight percent of the analyses required a change point in the model; the most common change points were 1987 and 1991. Of 169 total analyses, 63% showed a significant decrease, 6% showed a significant increase and 31% showed no significant pattern over time, i.e. slope did not differ from zero. Increasing contaminant concentrations for at least one compound were found in Lakes Huron, Michigan, Ontario and Superior. In a second temporal analysis, recent trends for SUM PCB and DDE were assessed by single line regression over three time periods: 1988-1993, 1989-1993 and 1990-1993. In 89% of the analyses (n=78), levels showed no statistical change, 5% showed a decrease and 6% showed a significant increase. Rising levels were found in Lakes Huron, and Superior; decreasing levels were found in the Niagara and Detroit rivers and Lake Erie. Spatial analysis on data from the last 5 yr indicated that oxychlordate, dieldrin and heptachlor epoxide had their greatest concentrations at Lake Michigan sites, DDE and SUM PCBs were greatest at Saginaw Bay, Lake Huron and mirex was most elevated in Lake Ontario.

AQUATIC PATHOLOGY AND ITS ROLE IN FORENSIC SCIENCE
SESSION CO-CHAIRS: C.G. ROUSSEAUX AND R. MULLER

INTRODUCTION TO THE ROLE OF PATHOLOGY IN AQUATIC TOXICOLOGY

C.G. Rousseaux
GlobalTox, 301 Metcalfe Street, Ottawa, ON, K2P 1R9

No abstract available.

MAXIMIZING RESULTS FROM PATHOLOGICAL INVESTIGATIONS

R. Muller
Health Canada, Tunney's Pasture, Ottawa, ON K1A 0L2

No abstract available.

DISEASES OF AQUATIC SPECIES IN THE MARINE ENVIRONMENT

G. Johnson
Atlantic Veterinary College, 550 University Avenue, Charlottetown, PEI, C1A 4P3

No abstract available.

STATISTICS IN AQUATIC TOXICITY TESTING WORKSHOP
SESSION CHAIR: B.A. ZAJDLIK

This session was an open forum organized by Dr. Zajdlik.

POSTER PRESENTATIONS/SÉANCES AFFICHES

VARIATIONS OF SCOPE FOR GROWTH IN *Mytilus edulis* AND *Mya arenaria* AFTER A CHRONIC EXPOSURE TO TRIBUTYLTIN

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Abstract

Antifouling paints are the major sources of tributyltin (TBT) in aquatic ecosystems. Several studies on deleterious effects of TBT on species like mussels or clams have been done but still, there is a lack of information about chronic toxicity of TBT and its transfer through the food chain. Our main goal is to evaluate the variations of scope for growth after a chronic exposure to TBT in M. arenaria and M. edulis. We worked in mesocosms and the uptake of TBT in bivalves was from food. Over a period of 3 months, physiological changes were monitored monthly as well as TBT tissue accumulation. TBT tissue accumulation was observed in both species. After a period of 3 months, we obtained an accumulation of 28 ngTBT/g w.w. in M. edulis and in M. arenaria a total of 133 ngTBT/g w.w. was observed. Our results on scope for growth differ for the two species. In the contaminated mesocosm, M.edulis had no reduction in scope for growth when compared with controls; however for M. arenaria, there was a reduction in scope for growth for the last 2 months. These results show that there could be an effect of TBT on scope for growth in M. arenaria

Introduction

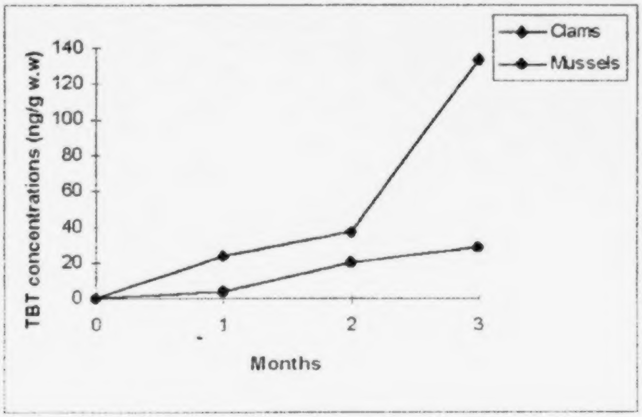
The use of tributyltin in antifouling paints is the major source of this contaminant in aquatic ecosystems. Several deleterious effects have been observed in molluscs and seem to be correlated with the high concentrations of tributyltin measured in marinas and harbours. It has been observed that there was impose in *Nucellar lapillus* and *Hinia reticulata* at a concentration of about 10 ngTBT/l seawater (Stroben et al, 1992). A long term study on the Pacific oyster (*C. gigas*) showed that there was abnormal shell thickening and weight loss at a concentration of 0,02 ugTBT/l seawater (Thaïn et al, 1987). Also it was observed by Beaumont and Budd (1984) that there was high mortality over a period of 15 days for the larvae of *M. edulis* at a concentration of 0,1ugTBT/l seawater and that the surviving larvae were moribund. As a polar hydrophobic compound, TBT can be transported through lipid membranes. Once inside the cytochrome P-450 dependent mono-oxygenase system hydroxylates TBT to β-γ-Δ-hydroxydibutyltin derivates. This derivate can be conjugated with sugars or sulfates and be transformed into a highly polar conjugate that is rapidly eliminated from the animal. The accumulation of TBT in molluscs is caused by the high n-octanol-water partition coefficient of TBT and also by the low cytochrome P-450 content and mixed-function oxygenase activity in the digestive gland of bivalves. Several studies on adverse effects of TBT on molluscs have been done but still there is a lack of information about chronic toxicity of TBT and its transfer through the food chain. Our objectives were to evaluate the bioaccumulation of TBT and the variations of scope for growth in *M. edulis* and *M. arenaria*. To achieve these objectives, we worked in experimental mesocosms and monthly, we monitored physiological changes as well as TBT tissue accumulation.

Materials and methods

We worked in experimental mesocosms located at Pointe-au-Père in Rimouski and the uptake of TBT in bivalves (*M. edulis, M. arenaria*) was from food (*Phaeodactylum tricornatum*). The experience lasted 3 months from July 1994 to September 1994. Over a period of 3 months physiological changes (filtration, excretion respiration and assimilation) were monitored monthly as well as TBT tissue accumulation. We also measured the O/N ratio as well as the scope for growth because they are important physiological indices. The scope for growth represents the energy balance of an animal under given conditions and the O/N ratio provides an indication of the balance between the rates of catabolism of protein, carbohydrate and lipid substrates in the animal's tissues.

Results and discussion

After 3 months, we obtained an accumulation of 28 ngTBT/g w.w. in *M. edulis* and in *M.arenaria* a total of 133 ng TBT/g w.w. was observed (Graph 1).



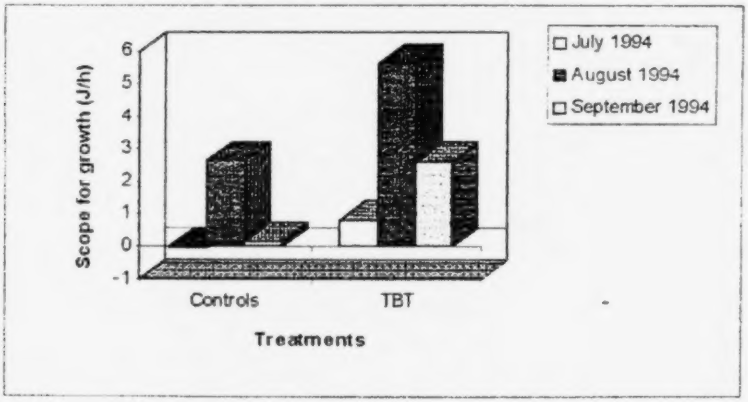
Graph 1: Bioaccumulation of TBT in *Mytilus edulis* and Mya are after a chronic exposure.

With the TBT to DBT ratio, we observed that under the same conditions, the ratio was 2 times higher for *M. arenaria* than for *M. edulis*. This could mean that *M. edulis* metabolise TBT more efficiently and also would explain the lower tissue accumulation in this species. Our results on scope for growth differed for the two species (Table 1).

Table 1: Physiological measures of Mytilus edulis and Mya arenaria obtained in July, August and September 1994 from the control and contaminated mesocosms.(* significant differences between the two treatments)

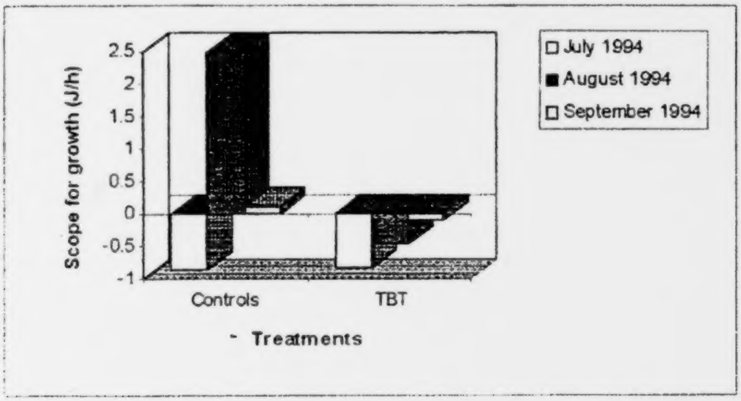
	CONTROLS					
	M.edulis			*M. arenaria*		
	July 1994	August 1994	September 1994	July 1994	August 1994	September 1994
Filtration rate (ml H_2O/g.h)	393	534	992	67*	139	234*
Excretion (µg NH_4-N/g.h)	8.8	3.5	3.01	3.99	2.2	2.63
Respiration (ml O_2/g.h)	0.024	0.018*	0.018*	0.011	0.007	0.007
Assimilation (%)	81	59	77*	68	77	87
O/N ratio	7.5	14.6	10.5	4.4	6.8*	10.5
Scope for growth (J/h)	-0.035	2.63	0.16	-0.852	2.47*	0.1
	CONTAMINATED					
	July 1994	August 1994	September 1994	July 1994	August 1994	September 1994
Filtration rate (ml H_2O/g.h)	617	843	1101	191*	92	146*
Excretion (µg NH_4-N/g.h)	24.7	10.2	2.9	8.95	3.57	3.14
Respiration (ml O_2/g.h)	0.025	0.016*	0.012*	0.009	0.006	0.005
Assimilation (%)	54	74	63*	62	47	72
O/N ratio	9.8	18.9	11.6	15.9	26.7*	4.6
Scope for growth (J/h)	0.83	5.6	2.6	-0.829	-0.469	-0.096

There were no adverse effects in scope for growth for *M. edulis* for the 3 months of contamination which could mean that below 28 ngTBT/g w.w. there is no effect of TBT for this species (Graph 2).



Graph 2: Scope for growth in *Mytilus edulis* from the control and contaminated mesocosms for the months of July, August and September 1994.

However for *M. arenaria*, we obtained a reduction in scope for growth for the last two months during which period the tissue accumulation was higher than 36 ngTBT/g w.w (Graph 3).



Graph 3: Scope for growth of *Mya arenaria* from the control and contaminated mesocosms for the months of July, August and September 1994.

The reduction in scope for growth between the treatments is significant for the month of August. There could be an effect of TBT in *M. arenaria* when the tissue accumulation is higher than 36 ngTBT/WNW.w but before a pollution effect can be concluded, normal temporal variations in energetic reserves must be considered for a complete understanding of the physiological effects following TBT exposure. Widows and Page (1993) observed that there was an increase in the rate of oxygen uptake and that there was a reduction in the clearance rate and scope for growth in *M.edulis* with increasing TBT concentration. Our results obtained for *M. arenaria* show a reduction in filtration rate and scope for growth in August and September but we didn't observe any changes in the respiration rate which could mean that filtration rate is the most important physiological process in scope for growth.

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DETECTING GENOTOXIC ACTIVITY OF INDUSTRIAL EFFLUENTS WITH THE SOS CHROMOTEST MICROTITRATION PROCEDURE

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The SOS Chromotest, a cost-effective short-term bacterial genotoxicity screening assay, was performed to appraise its capacity for detecting the presence of soluble genotoxic activity in neat (unconcentrated) effluent samples (organic and inorganic chemical plants, metallurgical plants, pulp and paper mills, municipal wastewater treatment plants) and in several surface water samples. Out of 48 effluent samples tested, 37 (77%) elicited a significant induction of the *Escherichia coli* PQ37 SOS-response. Effluents from inorganic chemical plants and pulp and paper mills displayed the most potent responses, with and without metabolizing enzymes (S9 mix). The genotoxic activity of whole effluents subjected to a 5-d aeration treatment was shown to be generally as high as that of native (unaerated) samples, suggesting that soluble genotoxicants are stable and/or that genotoxic metabolites may be formed during aeration. This study suggests that the SOS Chromotest may be sufficiently sensitive to screen for the presence of soluble DNA-damaging agents in a wide variety of unconcentrated complex samples (industrial and municipal treatment plant effluents) and even in surface waters. Test results as well as an optimized procedure for undertaking the SOS Chromotest with environmental samples will be presented.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR BROWN TROUT VITELLOGENIN FOR USE IN THE DETECTION OF ENVIRONMENTAL ESTROGENS

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Vitellogenin (Vg) is a phospholipoprotein egg yolk precursor found in females of most oviparous vertebrates. Vitellogenin induction in male teleosts is known to result from exposure to xenoestrogens. Using antisera for brown trout Vg provided by Drs. Norberg and Haux (University of Goegbord, Sweden), we adapted an (ELISA) for *in vivo* Vg in brown trout (*Salmo trutta*). We will use the Vg bioassay to detect the estrogenic effects of effluents, contaminated sediments and suspect chemicals. Vg production in male fish was induced by intraperitoneal injection of 17-β estradiol. Harvested and purified Vg was used in an assay based on competition between soluble Vg and Vg adsorbed in microtitre wells for binding sites on rabbit anti-Vg antibody. Adsorbed Vg-antibody complexes were subsequently revealed by an alkaline phosphatase technique. The reaction intensity was measured as absorbance and used to quantify the amount of antibody bound to adsorbed Vg. The amount of bound antibody was inversely proportional to the amount of vitellogenin in the fish plasma. Preliminary results show that the assay's working range, corresponding to 80-20% binding, was 14-355 NG/mL, with 50% binding (IC_{50}) around 71 NG/mL. Calculated at 50% binding, the intra-assay variation was less than 8% (n=9) and the inter-assay variation was also less than 8% (n=4). The assay's sensitivity was 0.067 NG/mL and the limit of detection was 0.134 NG/mL. We will present the results of Vg bioassays for the presence of estrogen mimics in industrial effluent.

ASSESSING ECOSYSTEM DYNAMICS OF THE SLAVE RIVER (NWT) - STABLE ISOTOPE ANALYSIS, BIOASSAY TESTING, AND ECOLOGY OF BENTHIC POPULATIONS

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The Slave River Environmental Quality Monitoring Program was established to assess whether the water and fisheries in the Northwest Territories portion of the River were being impacted by the downstream transport of contaminants from industrial and agricultural sources. Stable isotopes were examined in the current study to assess food chain and trophic level dynamics and their impact on the fish in the Slave River. From an ecological point of view, stable ratios of carbon, sulphur, and nitrogen can be used in the analysis of food chain dynamics to determine, for example, the feeding and migration patterns of fish. From a toxicological point of view, these stable isotope ratios are important because identification of food sources and pathways can help explain contaminant transfer dynamics through the food chain. Results of the current study suggested that at least two significant food sources are indicated by the stable isotope of sulphur. Also, the carbon isotope ratios indicate that the food source is via different pathways. This may suggest that sources of contaminants contributing to high chemical body burdens could also originate from a range of pathways. Bioassays using *Daphnia magna* were conducted to assess potential toxicity of suspended sediment from the Slave River. Collection of suspended sediment was initiated in October 1990 and continued until January 1994. No mortality was evident with any elutriate concentration during the acute 48-hr experiments, including the 100% (vol/vol) elutriate. Similar elutriate concentrations were added to the Microtox bioluminescence assay. Over the various test periods, no light inhibition of the bacteria was noted. Benthic invertebrate communities were characterized in sediment in the Slave River in 1990 and 1991. The majority of benthic invertebrates occurred at very low levels and organisms previously used in biomonitoring studies (e.g. bivalve molluscs, large oligochaetes) were rare or absent. Over 90% of the invertebrates collected were chironomids or small oligochaetes.

THE LETHALITY OF PYRETHRINS TO THE LARVAE OF THE AMERICAN LOBSTER (*Homarus americanus*)

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Pesticide formulations containing pyrethrum extract are being used to treat salmonids for infestations of the copepod parasites, *Lepeophtheirus salmonis* and *Caligus elongatus* (sea lice). We have determined the acute lethality of one such formulation to four larval stages of the American lobster (*Homarus americanus*), a species of significant economic importance in eastern Canada. The formulation tested contains 0.06% pyrethrum extract and 0.6% piperonyl butoxide (a synergist). Stage I larvae are significantly less sensitive than stages II, III or IV (48-h LC_{50} = 4.42 µg/L). Stages III and IV are not significantly different in their response to pyrethrum extract (48-h LC_{50} = 1.39 µg/L (stage III) and 0.73 µg/L (stage IV)). Most studies using lobster larvae have shown the earliest larval stage to be the most sensitive to chemicals. Our results show that the earliest larval stage is the least sensitive to this formulation.

LABORATORY AND FIELD TRIALS TO DETERMINE THE EFFECTS OF PYRETHRINS ON NON-TARGET INVERTEBRATES DURING TREATMENT OF ATLANTIC SALMON AGAINST SEA LICE

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Parasitic copepods have been a serious problem for the salmon aquaculture industry in Europe for several years and are now causing losses in the aquaculture industry in New Brunswick, Canada. A bath treatment (1 h) in a formulation containing pyrethrins and piperonyl butoxide has been recommended. Four invertebrate species: juvenile American lobster (*Homarus americanus*), adult shrimp (*Pandalus montagui*), green crabs (*Carcinus maenas*), and *Gammarus* sp. were tested to determine their relative sensitivity to the formulation. The two most sensitive species, lobster and shrimp, were then placed in small cages around a salmon aquaculture pen during an anti-louse treatment. Mortality of test animals was monitored over a 24-h period. Water samples were collected during and after the treatment, salmon were sacrificed and tissue collected for analysis. No evidence of treatment chemical was found in the water samples outside the treatment pen. The level of treatment chemicals in treated fish was very low. The level of mortality of test animals was not significantly different than that noted near control pens. The results indicate a low impact of this formulation on non-target invertebrates near salmon pens.

Overestimation of Toxic Equivalency Factors (TEFs) Resulting from Inhibition of EROD Activity by Cytochrome P450 1A Inducers in Cultured Cells

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Chlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls belong to a large group of ubiquitous environmental contaminants known as the halogenated aromatic hydrocarbons (HAH). Certain members of this group, in particular the planar HAH (PHAH), are highly toxic due to their high-affinity interaction with a receptor protein—the aryl hydrocarbon (Ah) receptor—and subsequent changes in gene expression leading to the disruption of processes involving the control of cell growth and differentiation (Safe 1990; Hankinson 1995).

There are large differences among the various PHAH in their affinities for the Ah receptor and, consequently, in their biological potencies. In an attempt to deal with the multiplicity of PHAH compounds and potencies and to express the potential biological activity of complex mixtures of PHAH, a toxic equivalency concept has been developed (Safe 1990). In this approach, the biological or toxic potencies of PHAH are expressed relative to a benchmark PHAH, usually 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Using a variety of endpoints or responses, a relative biological potency or "toxic equivalency factor" (TEF) can be determined for each PHAH, and the TEF values can be used in conjunction with data on the concentrations of the individual PHAH to calculate the "dioxin (TCDD) equivalents" (TEQ) in a particular environmental sample.

There is increasing recognition of taxon-specific differences in the relative potencies of individual PHAH, and several model systems are being developed and used to describe and quantify these differences (Gooch *et al.* 1989; Walker and Peterson 1991; Hahn *et al.* 1993; Kennedy *et al.* 1993; Clemons *et al.* 1994; van der Weiden *et al.* 1994; Hahn *et al.* 1995; Kennedy *et al.* 1995a; Kennedy *et al.* 1995b; Newsted *et al.* 1995; Parrott *et al.* 1995). Many of these systems use the induction of cytochrome P450 1A1 (CYP1A1) *in vivo* or in cultured cells to determine taxon-specific relative potencies of Ah receptor agonists and to assess TEQs present in complex environmental mixtures. Most such studies use the measurement of CYP1A1-dependent catalytic activities such as ethoxyresorufin O-deethylase (EROD) as a surrogate for the amount of induced CYP1A1 enzyme present. Current methods that use the EC_{50} for EROD induction as a measure of relative potency assume: 1) that EROD activity always reflects CYP1A content, 2) that all PHAH induce CYP1A/EROD with equal efficacies (maximal level of induced protein), 3) that dose-response curves for different PHAH are parallel (i.e. that ratios of potencies are the same regardless of the level of response being considered). Recent studies carried out in a variety of species and using both *in vivo* and *in vitro* systems show that these assumptions are frequently not supported by the data. For example, Parrott *et al.* (1995) found that dose-response curves for induction of EROD activity by a series of PCDDs and PCDFs in rainbow trout were not parallel. A further example can be found in the early studies of Sawyer and Safe (1982), who found large differences in the maximal level of EROD activity induced by various PCB congeners in rat hepatoma cells. Similar differences in maximal EROD responses have since been observed in other cell systems (Hahn *et al.* 1995; Kennedy *et al.* 1995b), suggesting that some PHAH may act as partial agonists (compounds with a lower intrinsic efficacy (Kenakin 1993)).

We have been developing an *in vitro* system utilizing a fish hepatoma cell line (PLHC-1 (Hightower and Renfro 1988)) for examining the relative potencies of PHAH in fish. In our initial studies, we noticed a discrepancy between dose-dependent patterns of EROD activity and immunodetectable CYP1A protein induced by the planar PCB congener 3,3',4,4'-tetrachlorobiphenyl (Hahn *et al.* 1993). Dose-response curves for EROD activity were biphasic, with strong induction at lower doses followed by a reduced response at higher doses. Such biphasic curves have been seen in a number of mammalian, avian, and piscine systems utilizing CYP1A activity as an endpoint (reviewed in Hahn *et al.* 1993). In contrast to the EROD data, levels of CYP1A protein continued to increase with increasing dose.

Recently, we conducted a series of experiments to examine dose-response relationships for induction of CYP1A catalytic activity (EROD) and CYP1A protein in PLHC-1 fish hepatoma cells, using a larger group of PHAH, including TCDD, 2,3,7,8-tetrachlorodibenzofuran (TCDF), and four planar chlorobiphenyl (PCB) congeners: 3,3',4,4',5-pentachlorobiphenyl (BZ# 126), 3,3',4,4',5,5'-hexachlorobiphenyl (BZ# 169), 3,3',4,4'-tetrachlorobiphenyl (BZ# 77), and 3,4,4',5-tetrachlorobiphenyl (BZ# 81) (Hahn 1994; Hahn *et al.* 1995). The cells were grown, treated, and assayed in multi-well plates, using methods developed by Kennedy (Kennedy *et al.* 1993; Kennedy *et al.* 1995a). In our studies, the individual PHAH differed in potency and apparent efficacy for EROD induction. In each case, EROD induction was biphasic, with stronger induction at lower concentrations and an attenuated response at higher concentrations. In contrast, the content of immunodetectable CYP1A protein increased monotonically with dose of PHAH, and the maximum level achieved was similar for all inducers. 2,3,3',4,4'-Pentachlorobiphenyl (BZ# 105), 2,3',4,4',5-pentachlorobiphenyl (BZ#118), and 2,2',3,3',4,4'-hexachlorobiphenyl (BZ# 128), *ortho*-substituted PCBs that induce CYP1A in mammals, were inactive as inducers of EROD or CYP1A protein in PLHC-1 cells, providing further evidence for relative potency differences between fish and mammals (M.E. Hahn, unpublished results).

Additional studies have shown that TCDF and 3,3',4,4'-TCB are competitive inhibitors of EROD activity in fish hepatic microsomes (Gooch *et al.* 1989; M.E. Hahn, unpublished results), suggesting a mechanism for the biphasic EROD response. We hypothesize that, at low concentrations, inducers bind to the Ah receptor and initiate transcription of the *CYP1A* gene, leading to an increase in CYP1A protein. At higher concentrations, residual inducer present in the cells binds to the CYP1A protein and inhibits CYP1A catalytic function, leading to reduced EROD activity despite the presence of elevated CYP1A protein. Thus, full agonists may appear to be partial agonists when only EROD activity is measured. The peak EROD activity will depend on the relationship between inducing potency and inhibitory potency, as shown in Fig. 1. The important consequence of this inhibition is that the maximum CYP1A induction response will be underestimated, leading to lower *apparent* EC_{50} values and thus the *overestimation* of relative potencies or TEFs for many inducers (Fig. 1) (Hahn *et al.* 1995).

These studies demonstrate the necessity of measuring both EROD activity and immunodetectable CYP1A protein for the accurate assessment of CYP1A induction and relative potencies, both in cultured cells (Hahn *et al.* 1995) and *in vivo* (Gooch *et al.* 1989). These findings further suggest the need for alternative dose-response models for estimating true EC_{50}s and relative potencies in cell culture bioassays.

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ADVOCATING THE ROUTINE MEASUREMENT OF CRITICAL BODY BURDENS (TISSUE RESIDUE LEVELS) IN STANDARD TOXICITY PROTOCOLS FOR USE IN REGULATORY PROGRAMS

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Body burden is the total amount of a substance an organism has taken up from all sources over time and retained in the body. Critical body burden (CBB) is defined as the minimum concentration that causes an adverse effect on the measurement endpoint (e.g., reproductive potential of *Daphnia*) of interest. Traditionally, results from acute and chronic toxicity tests are expressed in terms of the concentration in the external medium associated with the biological response of interest. Measuring the levels of a substance (CBB) in organisms during bioassays reduces the uncertainty associated with the determination of inherent toxicity (i.e. uncertainties related to the comparability of bioavailability, exposure routes and intake rates between the field and laboratory are no longer of concern). We feel that this information is needed by regulatory programs to further refine the effects side of an ecological risk assessment. There has been considerable interest generated in the theory of using CBBs but, as yet, there is little movement in adding this measurement to standard bioassay protocols. This poster will present the concept of critical body burden, its proposed use in regulatory programs, review national and international activities regarding CBB, and discuss its advantages and limitations in relation to traditional bioassay approaches.

USE OF MICROCOSMS TO VALIDATE AN ECOTOXICOLOGICAL RISK ASSESSMENT PROCEDURE

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A novel probabilistic risk assessment procedure may more accurately reflect the risks to an ecosystem then currently used methods. The model is data driven and provides environmental concentrations of toxicants that will protect a certain percentage of the species present, e.g. 95%. The present project focuses on the validation of a probabilistic risk assessment procedure using microcosms. Laboratory acute and chronic data for several species were plotted against the species susceptibility and environmental concentrations of Guthio (azinphos methyl). The field study attempts to validate the hypothesis that laboratory data can be used to predict field results based on the probabilistic model. These artificial pools were dosed with five different concentrations (0.01, 0.1, 1, 10, 200 µg/L) of the organophosphorous insecticide azinphos methyl (Guthion) in the first year of the study. The range of test concentrations encompasses a sub-chronic to acute toxicity level. The microcosms, each 12 m^3 in volume, will contain: sediment placed in trays that cover 50% of the area, several pots of the macrophyte Myriophyllum, periphyton, and fish were among the test organisms. A pretreatment period of 4 wk provided time to minimize variability among ponds with circulation of water from the control pond, as well as natural colonization of the microcosms. Treatment with Azinphos methyl was in the form of a solution of an emulsifiable concentrate sprayed into the ponds. Under the expected conditions of pH and temperature the pesticide has a half life of about two days and concentrations were replenished accordingly. Monitoring was conducted on parameters including community structure, function and water residues among others.

VALIDATION AND SENSITIVITY COMPARISONS OF MICRO-SCALE TOXICITY TESTS FOR THE EVALUATION OF FRESHWATER SEDIMENT TOXICITY

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A 3-yr study is currently underway to develop a representative and cost-effective battery of toxicity tests for evaluating freshwater sediment and pore-water toxicity. Among the tests currently being evaluated are the following: Microtox™ chronic test, Microtox™ solid-phase test, Microtox™ liquid phase test, Thamnotoxkit F™, Rotoxkit F™, *Daphnia magna* IQ test™, Sediment Toxkit, SOS Chromotest, a *Selenastrum capricornutum* short exposure assay, and trout hepatocyte assays. Conventional sediment tests with *Chironomus tentans, Hyallela azteca* and *Lumbriculus variegatus*, as well as benthic macroinvertebrate community assessments and sediment chemical characterizations, are being conducted at two contaminated sites. Toxicity test reproducibility, sensitivity, practicality, cost and ecological relevance are discussed.

MARINE ECOTOXICOLOGY WITH ECHINODERM LIFE CYCLE

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Only a few scientists have compared the effects of chemicals on various life stages in the sea urchins. Throughout my works, it is noted that the sperm is the most sensitive link in the success of fertilization and subsequent development. According to the other workers, concentrations of crude oil do not affect fertilization and development of some urchin embryos, whereas many oil dispersants have more or less serious influence on the development of the skeleton and the endoderm in the embryos. Heslinga (1976) tested the effects of Cu in seawater on the fertilization success, early cleavage, larval skeletal development and survival of adults of the sea urchin, and concluded that the later developmental stages appear to be very sensitive and may therefore be the most suitable stages for assessing toxic effects in the sea urchin.

In my experiments with sea urchin eggs, sperm is the most sensitive, egg is the next, fertilization and cleavage are more sensitive to Cu than in Heslinga's experiment; later developmental stage is more sensitive and adults are, on the contrary, less sensitive than the earlier stages. In details, the sensitivity of developmental processes to chemicals on sea urchins possibly demonstrates an order of sensitivity, sperm + egg > sperm > egg > gastrula, metamorphosis > fertilization > blastula, pluteus > cleavage > adult (see Table).

Table. Estimated threshold concentrations (in ppm) of Cu, Zn, ABS and NH_4 associated with a reduced development in sea urchin eggs. The time of exposure with respect to fertilization and/or exposure duration is given between brackets.

Species/ chemical	Temp. (°C)	Sperm (before fertil.) (-2-3 min)	Eggs (before fertil. (-3-6 h)	Sperm/eggs before fertil.) (-2-3 min -3-6 h)	Ferti- lization (0 min)	First cleavage	Blastula	Gastrula	Pluteus	Further stages metamor phosis
Peronella japonica	26					(40 min)	(8 h)	(12 h)	(24 h)	(4 d)
$CuSO_45H_2O$		0.005			0.01	0.02	0.02	0.005	0.02	0.01
$ZnCl_2$		0.005			0.01	0.02	0.05	0.005	0.05	0.01
ABS		0.1			0.3	1.0	1.0	0.5	0.5	0.2
NH_4Cl		0.2			0.5	1.0	1.0	0.5	0.5	0.2
Heliocidaris erythrogramma	24					(70 min)	(10 h)	(15 h)		(2.5 d)
$CuSO_45H_2O$		0.001			0.01	0.02	0.01	0.005		0.005
Hemicentrotus pulcherrimus	17					(90 min)	(12 h)	(24 h)	(48 h)	1 yr adult (72 h)
$CuSO_45H_2O$		0.01	0.01	0.01	0.05	0.1	0.1	0.05	0.05	1
$ZnCl_2$		0.02	0.02	0.01	0.05	0.1	0.2	0.05	0.05	5
ABS		0.2	0.5	0.3	1.0	2.0	2.0	1.0	0.5	100
NH_4Cl		0.5	0.5	0.2	1.0	2.0	1.0	0.5	0.5	100
Anthocidaris crassispina	26					(45 min)	(5 h)	(12 h)	(24 h)	
$CuSO_45H_2O$		0.02	0.02	0.01	0.05	0.1	0.1	0.05	0.02	
$ZnCl_2$		0.02	0.05	0.01	0.05	0.1	0.1	0.05	0.02	
ABS		0.5	0.5	0.3	1.0	5.0	2.0	1.0	1.0	
NH_4Cl		1.0	1.0	0.5	2.0	5.0	5.0	2.0	1.0	
Pseudocentrotus depressus	17					(90 min)	(10 h)	(19 h)	(36 h)	
$CuSO_45H_2O$		0.015			0.03	0.06	0.06	0.03	0.02	
ABS		0.5			1.0	2.0	2.0	1.0	1.0	

METHODOLOGY FOR RISK ASSESSMENT OF PETROLEUM HYDROCARBON CONTAMINATION IN THE AQUATIC ENVIRONMENT

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Petroleum hydrocarbons encompass a diverse group of organic chemicals, each with different chemical structures, physical-chemical properties and toxicity. Unrefined and refined petroleum products, such as fuels and oils, are complex mixtures of various organic hydrocarbons. Environmental contamination with petroleum products is typically identified by chemical analysis of the bulk sum parameter, TPH (total petroleum hydrocarbons). While TPH is considered to be a reliable indicator of petroleum hydrocarbon contamination, this parameter provides no information on the potential toxicity or environmental impact of the contamination, since toxicity is determined by the specific chemical constituents of the petroleum mixture.

In order to assess the potential risks to aquatic life from exposure to TPH, various hydrocarbon fractions of TPH were grouped based on chemical structure-activity relationships. Next, appropriate water and sediment quality guidelines for the protection of aquatic life were compiled. For chemicals lacking appropriate water and/or sediment guidelines, the aquatic toxicity literature was reviewed to determine adverse or no effect levels in various aquatic species.

Surrogate chemicals were selected based on the available aquatic toxicity data for individual TPH chemicals, appropriate water and sediment quality guidelines for the protection of aquatic life, general structure activity relationships, and solubilities of various chemical components of TPH, identified through analytical scan of the organic hydrocarbon fractions.

The selected surrogates are:

- n-decane for the linear and cyclic alkanes
- phenol for the phenolics and substituted phenolics
- toluene for the substituted benzenes
- benzo[a]pyrene for the carcinogenic polyaromatic hydrocarbons (PAHs)
- phenanthrene for the non-carcinogenic hydrophobic PAHs
- naphthalene for the non-carcinogenic hydrophilic PAHs

Water and sediment concentration limits for each surrogate chemical/ chemical group are proposed for the assessment of the risk of adverse effects to freshwater aquatic life.

This approach for evaluating the toxicity of individual sub-groups of TPH can be applied to risk assessments of petroleum hydrocarbon contaminated sites. GC/MS open characterizations are conducted for sediment and water samples and detected chemicals are grouped into their respective sub-groups of TPH. The relative percentages of each sub-group of the TPH mixture are calculated. Risks of adverse effects to aquatic life are estimated by calculation of concentration ratios for each sub-group of TPH. Concentration ratios that exceed one indicate possible risk to aquatic organisms from that particular sub-group of petroleum hydrocarbon chemicals. Remedial objectives can be established for TPH based on the relative percentage and toxic potency of the TPH sub-groups.

There are many data gaps and research needs associated with the above methodology. The existing water and sediment toxicity data for TPH chemicals consists mainly of acute, static bioassays using nominal concentrations. In addition, most of these studies were conducted several years ago. The following is a partial list of the key areas requiring further study for the assessment of petroleum contaminated sites:

- more early life stage chronic toxicity studies
- high quality sediment toxicity studies from which to derive acceptable concentration limits

A preliminary approach for the ecological assessment of petroleum contaminated sites is proposed based on the available toxicological data for freshwater species. While there are several areas which require more research, the proposed approach offers an effective strategy for risk assessment of TPH contamination in the aquatic environment.

CONCENTRATIONS OF PAH'S IN WHITE SUCKERS (Catostomus commersoni) FROM THE St. LAWRENCE RIVER, NEAR CORNWALL, ONTARIO

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The purpose of this study was to compare concentrations of polycyclic aromatichydrocarbons (PAH's) in the muscle tissue of white suckers (Catostomus commersoni) collected from the St. Lawrence River upstream and downstream of Cornwall,Ontario/Massena, New York, and to relate these concentrations to differences in population characteristics between the two regions. Concentrations of 17 priority PAH's and 17 alkyl substituted derivatives were analysed in 80 white suckers (40 upstream from 8 sites, 40 downstream from 9 sites) using GC-MS-SIM techniques. Concentrations of mean total PAHs were significantly higher upstream (75.2 ng/g dry wgt. Std. Dev.=30.4) than downstream (53.6 ng/g dry wgt. Std. Dev.=29.7). White suckers from upstream in comparison to downstream were significantly shorter and weighed less at older ages (ages 5 to 11 for females, ages 6 to 11 for males), had a lower average fecundity, a greater mean egg diameter, higher condition factors, greater mean age, a greater lip and body papilloma incidence, and a lower catch per unit effort. There was no significant relationship found between the concentration of PAHs in the muscle tissue and the length,weight, age, sex, fecundity, mean egg diameter, GSI, condition factor, or lipidcontent of a fish. Therefore, the differences noted in population characteristics between the two regions could not be attributed to differences in PAH exposure. Although, the increased incidence of lip and body papillomas upstream may be related to PAH exposure. It is likely that there are a number of different contaminants stressing the white sucker populations of the St. Lawrence Riverand these will be investigated upon further study.

PAH BINDING TO ORGANICS PRESENT IN NATURAL WATERS AND PULP MILL EFFLUENTS

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Fluorescent quenching was used to measure the binding of selected polyaromatic hydro-carbons (phenanthrene, anthracene, pyrene, benzo[*a*]pyrene and benzo[*a*]anthracene) to isolated dissolved organic carbon fractions found in pulp mill effluents and a natural marsh. Organics were isolated by reverse osmosis and ultrafiltration. The natural marsh DOC (concentrated by reverse osmosis) was further fractionated into two molecular weight classes by ultrafiltration. The low MW organic substances usually gave lower binding constants. The organic substances isolated from pulp mill effluent had similar Kdoc values for phenanthrene and anthracene, but were 3- to 9-fold higher for pyrene, benzo[*a*]anthracene and benzo[*a*]pyrene. The DOC from two of the pulp mill sources were isolated by tangential flow filtration and would be higher molecular weight than the marsh DOC. Untreated commercial Aldrich humic acid gave similar results to the pulp mill samples, but gave lower binding constants when extracted with methanol. The fluorescent spectra of the various DOC sources showed interesting differences, but preliminary observations showed no correlation with PAH binding constants.

MEASUREMENT OF PARAMETERS AFFECTING THE ATMOSPHERIC FLUXES OF PAHs ACROSS THE AIR/WATER INTERFACE

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Long-range atmospheric transport is the major route for transport of PAHs to and from bodies of water; and fluxes can take place in three major forms; wet and dry deposition which can be measured directly, and gas exchange which can be estimated using the contaminant Henry's Law Constant and Mass Transfer Coefficient. When available, these constants are from limited laboratory determinations often at room temperature which do not represent environmental conditions. The Henry's Law Constant is affected by environmental conditions such as temperature and salinity of water, and Mass Transfer Coefficients are not only affected by temperature but also by wind speed and wave conditions. The effect of water temperature and salinity on Henry's Law Constant for naphthalene, phenanthrene, and anthracene was measured using a thermostated, on-line sparging column. Wind and wave effects on mass transfer coefficient for naphthalene were measured in a 32 m recirculating gas transfer flume under different wind and wave conditions. Results from these experiments along with appropriate functions to paramertized gas exchange will be presented.

TOXICITY IDENTIFICATION EVALUATION OF SUSPENDED SEDIMENTS FROM THE ATHABASCA RIVER

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Previous toxicity studies on sediment and fish from the Athabasca River suggest that naturally occurring substances within the oil sands area are responsible for the toxic effects. A Toxicity Identification Evaluation (TIE) study on dichloromethane (DCM) extracts of suspended sediment was undertaken to isolate and identify these compounds. The toxicity was determined by the standard Microtox^R test. Base/neutral and acid partitioning of the DCM extract shows toxicity in the base/neutral fraction only. Chromatographic fractionation of the base/neutral extract on silica-diol columns with DCM, DCM:methanol and methanol shows toxicity in the DCM fraction. This toxicity correlates with the presence of molecular sulfur as determined by gas chromatography-mass spectrometry. The toxicity of co-extracted sulfur to Microtox^R has been reported (Jacobs et al. 1992). Work is in progress to determine the relative contribution of molecular sulfur to the toxic effects. Also in progress is a comparison of the Microtox^R solid-phase test with solvent extracts of sediments from the Athabasca River.

PENETRATION AND EXTRACTABILITY OF PESTICIDES INTO AND FROM PLASTIC USED FOR CONTAINER MANUFACTURE AND RECYCLING

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High density polyethylene, HDPE, is used extensively in the manufacturing of pesticide containers. Because containers are not suitable for reuse, there has been an initiative to recycle these plastic containers into potentially useful products. However, there is measurable pesticide dislodgeability and leachability from these products. Evidence suggests that pesticides, particularly solvent based formulations, can penetrate into the plastic matrix of containers during storage. A methodology was developed to quantitatively analyze pesticide

penetration, and also determine formulation and container combinations which would minimize penetration. Five ^{14}C-labelled, commercial pesticide formulations were used to examine penetration into HDPE and fluorinated HDPE containers. Plastic disks taken from containers were clamped to the top of crimp-cap septum vials containing the radiolabelled pesticides. Vials were inverted and stored at different temperatures and time periods. At the end of a specified storage period, the disks were oxidized, and radioactivity ($^{14}CO_2$) was determined to assess the degree of pesticide penetration into the plastic. The container recycling process was also simulated to determine how much, and what type of pesticides could potentially be extracted from recycled products. HDPE was melted and mixed with a known quantity of radiolabelled pesticide. Shaved sections of the solidified and mixed HDPE were subjected to solvent extractions, and extracted pesticide residues were quantitated using scintillation counting. Residues remaining in the shaved sections were quantitated after oxidation. These methodologies could be utilized to provide quality control for container recycling and aid in the estimation of environmental and human risk.

TRENDS IN ORGANOCHLORINE CONTAMINANTS IN SEABIRD EGGS FROM ATLANTIC CANADA, 1968-1992

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Organochlorine contaminants were measured in eggs of three seabird species from 1968 to 1992 to monitor marine environmental quality and assess possible implications for seabird health. Eggs were collected at four-year intervals from eastern Canadian colonies of double-crested cormorant (*Phalacrocorax auritus*), Leach's storm-petrel (*Oceanodroma leucorhoa*) and Atlantic puffin (*Fratercula arctica*). DDE and PCB levels have decreased in all species at all colonies since 1972. DDE and PCB levels were higher in the Bay of Fundy and St. Lawrence Estuary than off Newfoundland, and were highest in cormorants and lowest in puffins. Hexachlorobenzene levels, in contrast, have not declined steadily since 1972, and are highest in puffins and lowest in cormorants. Temporal, geographic and interspecific trends will be presented for other organochlorines including dieldrin, oxychlordane, hexachlorocyclohexane and mirex. Contaminant patterns and trends will be interpreted, considering the historical use of the contaminants and their fate, the seabirds' foraging habits, and the proximity of colonies to different sources. Implications for seabird health and marine environmental quality will be discussed.

MERCURY LEVELS IN GREAT LAKES HERRING GULL EGGS, 1972-1992

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Since 1971, the herring gull (*Larus argentatus*) has been used as a sentinel species for monitoring the levels of persistent contaminants in the Great Lakes ecosystem. In this study, 21 herring gull colonies in the Great Lakes and connecting channels were sampled for years 1972-1976, 1981-1983, 1985 and 1992. For each year, 10 eggs (usually) were collected from each colony site and analyzed for total mercury (mg/kg, wet weight). Results indicated that eggs from Lake Ontario displayed the highest mercury levels (0.28-0.73 mg/kg), followed by Lake Superior (0.21-0.48 mg/kg). Lake Erie typically displayed the lowest mercury levels (0.18-0.24 mg/kg). Overall, mercury levels ranged from 0.12 in 1985 to 0.88 mg/kg in 1992 for Channel/Shelter Island (Lake Huron) and Pigeon Island (Lake Ontario), respectively. Generally, all colony

sites showed peak egg mercury levels in 1982. A significant decline in egg mercury levels was observed in six colony sites between 1972 and 1992 and in three colony sites between 1981 and 1992. The mean herring gull egg mercury levels observed in the early and mid-1970s and in 1982 for some colony sites were within the range found which potentially reduces hatchability in other fish-eating bird species.

MERCURY CONCENTRATIONS IN FEATHERS OF THE COMMON LOON

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The Common Loon (Gavia immer) is a high trophic level, long-lived, obligate piscivore at risk from elevated levels of anthropogenic mercury (Hg) through biomagnification. Atmospheric Hg is deposited in lakes throughout the loon's breeding grounds where it concentrates in fish, their primary prey. An effective technique for the capture of breeding loons now provides an opportunity to measure Hg exposure and its associated effects on survival, behavior, and reproductive success.

From 1991-1994 secondary feathers were removed from 374 adult loons in the upper Great Lakes Region and 20 in northern New England. Previous studies indicate that up to 93% of the body burden of Hg is deposited in feathers. Mercury found in the feathers is of the organic form, methylmercury. Total Hg (inorganic plus organic) was measured for the feather samples in this study. Feather Hg levels for adult loons range from 4.0 to 36.7 µg/g (dry weight) with a mean of 11.9 +/- 4.6 µg/g. Between-site variation (n=8 sites) is not significant (F=1.81, P>.05), although there is a marginally significant difference (P=.08) between Isle Royale, Michigan (lowest mean feather Hg, 7.6 µg/g) and New England (highest mean feather Hg, 13.3 µg/g). Feather Hg levels exceeding 15 ppm compose 17% of the sample set and in experiments with captive waterfowl, comparable levels of Hg were found to cause decreased reproductive success.

Potentially confounding variables associated with Hg exposure include gender, males (n=205) and 9.9 for females (n=182) and range from 20-37% higher in males.Gender differences are significant (F=49.7, P<.01). This is possibly due to maternal transfer of Hg and prey preferences. Male weights average 21% more than females (n=448). This sexual dimorphism may be related to forage niche partitioning. Age-dependent relationships with Hg levels may exist in Common Loons. From a sample of 62 adults, recaptured at least one year later, 47 (76%)exhibit an increase in Hg accumulation and 15 (24%) show a decline. The one-year Hg accumulation rate for males and females is equivalent (mean per year, n=35). The two-year Hg accumulation rate is 18.4% (n=14), although genders differ with rates of 30.0% in males and 12.7% in females.

The naturally occurring trace element Selenium (Se), may compensate or eliminate the toxic action of Hg and reduce its assimilation and rate of accumulation. As Hg increases, the effect and availability of Se progressively els range up to 8.6 µg/g with a meanof 4.4 +/- 0.2 µg/g (n=382). The molecular weight ratio between Se and Hg is 2.54, while the mean field ratio observed in loon feathers from this study is 2.72. This difference may represent an inability of loons to compensate for elevated levels of mercury. Males are more vulnerable to elevated Hg levels because 66% exhibit field ratios greater than the elemental ratio (versus 36% in females).

MERCURY DEPOSITION IN OMBROTROPHIC BOGS IN NEW BRUNSWICK, NOVA SCOTIA AND PRINCE EDWARD ISLAND

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Ombrotrophic or raised bogs receive all of their water and nutrients from the atmosphere and as such are well suited to record the chronology and magnitude of atmospheric deposition of heavy metals. Peat cores were collected from five raised bogs from remote areas in New Brunswick, Nova Scotia and Prince Edward Island. One core from each bog was dated using ^{210}Pb dating and corresponding sections from three cores from each bog were analyzed for total mercury content. Results were highly variable with no common pattern of atmospheric mercury deposition exhibited by all bogs. Three of the bogs showed a general trend of increasing mercury deposition from the mid-1800s (0.018-0.124 mg/kg dry weight) until the late 1970s and early 1980s (0.083-0.157 mg/kg), when mean mercury concentrations in peat dropped off markedly approaching pre-1850's values (0.035-0.051 mg/kg dry weight). Variability in the data may be the result of sampling methods, regional atmospheric deposition patterns or mobility of mercury within the peat profile.

A COMPARISON OF EXTRACTION AND AQUEOUS PHASE ETHYLATION FOR THE DETERMINATION OF METHYLMERCURY

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The solvent extraction technique for measuring organic mercury (Alli et al., 1994, J. High Res. Chrom., v.17) has emerged as a competing technique to the widely accepted aqueous phase ethylation technique (Bloom, 1989, Can. J. Fish. Aquat. Sci., v.46), but no comparisons have been made. We set out to compare the two methods for determining organic mercury in a variety of environmental media. For aqueous phase ethylation, distilled or digested samples are reacted with sodium tetraethylborate. The Hg compounds are ethylated, then purged with nitrogen onto TENAX traps. The traps are thermally desorbed in a stream of argon, and the Hg compounds are chromatographically separated on a 50 cm packed column, 15% OV3 on Chromasorb WAW-DMSC. The species are pyrolitically converted to elemental Hg, and quantified by cold vapour atomic fluorescence spectrophotometry (CVAFS). For the extraction technique, samples are treated with copper sulfate and acidic potassium bromide, extracted in dichloromethane, cleaned up in sodium thiosulfate and back extracted into dichloromethane. The extracts undergo GC analysis using a megabore column (15m x 1.5 micron DB-1 solid phase) with helium as the carrier gas. Hg compounds are pyrolyzed to elemental Hg and quantified by CVAFS.

Both techniques determine methylmercury in samples. The ethylation technique, however, does not allow for positive identification of other organic Hg compounds, due to the derivatizing reaction of the tetraethylborate. The solvent extraction technique does not derivatize species, so each organic Hg specie can be identified positively. In a series of 14 largemouth and smallmouth bass analyzed from Ranger Lake in south central Ontario, we found that methylmercury was typically the only organic Hg form present. However, in one of the 14 fish, ethylmercury was also present, but only as 5 % of the total organic Hg. The presence of ethylmercury in some environmental samples suggests that its presence should be searched for elsewhere, to determine its distribution and perhaps its source. Though toxicities of methyl- and ethylmercury are probably similar, their separate analysis is important in order to help regulate potential anthropogenic sources.

LEAD INTERACTIONS ON THE GILLS OF RAINBOW TROUT

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Rainbow trout (*Oncorhynchus mykiss*, 0.5-7.5 g) were exposed to 0.6 µM Pb (~125 µg/L Pb) for 2-3 h in soft water (Ca, Na ~45 µM; pH ~6.5). Complexing ligands (ethylenediamine, nitrilotriacetic acid, dissolved organic carbon) and competing solutes (Ca^{++}, Na^+, H^+) were added to the water. After exposure, gills were removed and analyzed for Pb using a graphite furnace atomic absorption spectrophotometer. From the data, a conditional equilibrium binding constant (*K*) for Pb-gill interactions was calculated to be log *K*~9.4, with about 130 nmol Pb-gill binding sites per gram of gill tissue. Conditional equilibrium constants were also calculated for Ca^{++}, Na^+, and H^+ binding to the Pb-gill binding sites. Experimentally determined log *K* values were entered into the MINEQL$^+$ aquatic chemistry equilibrium program, to predict binding of Pb on trout gills. Predicted values were compared to measured binding of Pb to gills of trout held in a series of natural waters with added Pb.

COBALT BINDING TO GILLS OF RAINBOW TROUT: AN EQUILIBRIUM MODEL

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Rainbow trout (*Oncorhynchus mykiss*, 1-5 g) were exposed to approximately 7.5 µM Co in synthetic soft water for 2-3 h at pH ~6.5. The water contained either complexing ligands such as ethylenediamine or competing agents such as Ca^{++}, Na^+, and H^+. After exposure, gills were analyzed for total bound Co on a graphite furnace AAS. From our complexation data, a conditional equilibrium binding constant (*K*) for Co-gill interactions was calculated to be approximately log *K*=5.9. Conditional equilibrium constants were also calculated for Ca^{++}, Na^+, and H^+ binding to the gill cobalt sites. These equilibrium constants were entered into an aquatic chemistry equilibrium program, MINEQL$^+$, to predict binding of Co to gills of fish held in a series of natural waters with added Co.

STUDY OF METAL INHIBITION IN THYLAKOID MEMBRANES USING SIMULTANEOUS FLUORESCENCE AND PHOTOACOUSTIC MEASUREMENTS

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Photosynthetic organisms, including phytoplankton, are strongly affected by metal pollution. One of the first mechanism inhibited by metals is, in fact, photosynthesis. In this process, the light energy absorbed by the photosynthetic apparatus is used to drive photochemical reactions (electron transport) which produce carbohydrate compounds. A part of this absorbed energy will be re-emitted in the form of fluorescence and non-radiative (heat) emission. All those mechanisms take place in the chloroplast, an organel constituted of membranes named thylakoids. Isolated thylakoids were used in this study since they are found in every photosynthetic organisms (from algae to vascular plants). The photosystem I and II are the mean constituents of thylakoid electron transport. Thus, the presence of electron transport inhibitors will change the balance between the mechanisms. The use of simultaneous fluorescence and photoacoustic (heat emission) measurements provides a non invasive method for studies of electron transport inhibition since no artificial electron donor or acceptor is needed. Fluorescence and heat emission procure information on the mode of inhibitory action of toxic metal cations in photosynthetic organisms.

CONTAMINANTS IN COPPER REDHORSE, AN ENDANGERED FISH SPECIES IN QUEBEC WATERS

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Copper redhorse (Catostomidae: *Moxostoma hubbsi*) is an endangered fish species whose world distribution is limited to a few tributaries of the St.Lawrence river. Severe reproduction failure and lack of successful recruitment have been reported for this rare species. As part of a multidisciplinary research program to protect this species from extinction, seven accidentally killed individuals from the Richelieu River (Québec) were analysed for trace metals, pesticides, PAH, PCB, dioxins and furans. Contrary to results obtained for other St.Lawrence fish species, dioxins were not detected (<0.00005 ng/g wet weight) in Copper redhorse and furans (T4CDF) were present at very low levels. Concentrations in PCB and organochlorine pesticides (mostly DDT derivatives, α-BHC, chlordane, dieldrine) were similar in livers and gonads. Coplanar PCB's (#77 and 126) were always detected, but coplanar 169 was not common. PAH's were virtually absent except for one specimen showing high levels of volatile compounds. Cadmium, lead, zinc and mercury were most frequently detected metals but Arsenic was never detected (<140 ng/g). These results indicate that the types and concentrations of contaminants in Copper Redhorse differ and are generally lower than those reported for benthic fish species from the St.Lawrence river. It is suggested that Copper Redhorse from the Richelieu River represent an isolated population which is relatively little exposed to St.Lawrence waters. Further research is necessary to assess the potential link between contaminants and reproductive failure in this fish species.

CHANGES IN ORGANOTIN CONCENTRATIONS OF WATER, SEDIMENTS AND MUSSELS IN THE ATLANTIC REGION FROM 1988 TO 1994

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The Canadian use of organotins as antifoulants on vessels was further restricted in 1989 due to evidence that such uses were producing environmental concentrations which had demonstrated adverse effects on gastropods and bivalve molluscs. Samples of marine water, sediments and blue mussels (*Mytilus edulis*) were obtained from various locations 1 yr prior to the implementation of those restrictions and analyzed for butyltin content. The locations sampled included large vessel (shipping) traffic and ship repair areas, as well as areas which were used by fishing and pleasure craft only. The results from that survey were compared with similar sampling conducted in 1994. While water concentrations were mostly below detection limits and mussel tissue concentrations did not change between 1988 and 1994, in 30 of the 35 locations sampled, sediment concentrations of total butyltin appeared to increase during that interval. The increases were noted in the fishing and pleasure-craft only areas as well as the large vessel traffic areas. In addition, the ratios of monobutyltin to tributyltin concentrations indicated the probability of fresh sources of TBT. The effectiveness

of the 1989 control in reducing TBT in the Canadian marine environment is obviously questionable.

HEAVY METALS IN YOUNG-OF-THE-YEAR ATLANTIC SALMON COLLECTED NEAR MINING ACTIVITY ON THE TOMOGONOPS AND NORTHWEST MIRAMICHI RIVERS, NEW BRUNSWICK

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To assess the fate and distribution of heavy metals in rivers influenced by metal mining activity, a study on the concentrations of heavy metals in young-of-the-year (YOY) Atlantic salmon and their food items has been initiated in the Miramichi River watershed. During October and November 1993, YOY Atlantic salmon and aquatic invertebrates were collected at sites near the mouth of the Tomogonops River and downstream on the Northwest Miramichi River. The Tomogonops River is influenced by the effluent and site drainage from a base metal mining operation at Heath Steele. Specimens collected were analyzed (whole-body) for a broad range of elements by ICP-MS. Concentrations of several elements in YOY salmon, including lead, cadmium, cobalt, manganese, selenium and thallium, showed an increase and then gradual decline downstream of the Tomogonops-Miramichi confluence. Aluminium, iron and lithium did not show any increase in concentration, but declined with distance downstream. Young-of-the-year Atlantic salmon are territorial and fast growing, which makes them good potential indicators of contaminants present in their habitat. Aquatic invertebrates form part of the food chain leading to fish in streams, and may provide an effective conduit for contaminants from periphyton, and particles in water, into fish.

THE TOXICITY OF ALUMINUM TO JUVENILE ATLANTIC SALMON (*Salmo salar*) IN SOFT, ACIDIC, ORGANIC SOLUTIONS

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Laboratory and field studies have shown that the toxicity of Al is reduced in the presence of dissolved organic matter (DOM). However, it appears that the residual toxicity is not always proportional to the levels of inorganic monomeric Al (Al_i) present. This disagrees with the predictions of the free ion model (FIAM) which suggests that the toxicity of a metal is determined by the concentration of the free or aquo ion - $M(H_2O)^{z+}$ or M^{z+}. For example, the FIAM has successfully explained the reduction in toxicity that occurs when metals are bound by inorganic ligands.

The aim of these experiments was to test the predictions of the FIAM model in a soft acidic water containing Al, with fulvic acid as a source of DOM. Juvenile Atlantic salmon (*Salmo salar*) were exposed to concentrations of Al in both inorganic and organic acidic soft waters, at pH 5.0. LC_{50}s were determined in the two solutions. Inorganic monomeric Al was measured spectrophotometrically after ion-exchange, and after equilibrium dialysis. If the FIAM applies, the LC_{50}, expressed as Al_i, would not differ in the two conditions at the same pH. Contrary to the predictions of the FIAM, the LC_{50}s were significantly different in inorganic and organic solutions. A regression model of the mortality data included terms for Al_i and DOM. Our data suggest that DOM not only decreases toxicity by complexation of Al, but also has an independent, protective role.

A STANDARD ARTIFICIAL MEDIUM FOR *Hyalella azteca*, INCLUDING THE ESSENTIAL BROMIDE ION

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Hyalella azteca is the most common and widely distributed freshwater amphipod in North America, and is becoming one of the most frequently used animals in aquatic toxicity testing. A completely defined artificial aqueous medium for this species was developed to allow standardization of toxicity tests through time and across laboratories, and to facilitate studies on pollutant interactions with specific water chemistry components. The importance of all aqueous ions was assessed. Sodium and bicarbonate are the most essential ions. Calcium and bromide are the only other 2 aqueous ions needed for long term (10 wk) survival when cotton gauze is present as a substrate and Tetra-Min fish food flakes are used as food. Calcium and bromide act synergistically; neither ion by itself improves survival. Magnesium and potassium improve growth, and potassium improves production of live young. Sulphate and chloride are not needed. A standard 5-salt artificial medium suitable for long term experiments and culture of *Hyalella* is proposed.

BEST STUDENT PRESENTATION AWARDS/
PRIX DES MEILLEURES PRÉSENTATION D'ÉTUDIANTS

Best Platform Presentations
top left D.W. Johnston
Department of Zoology, University of Guelph
top right J.E. Smits
Department of Veterinary Pathology
University of Saskachewan

Best Poster Presentation
opposite G.M. O'Brien
Centre for Toxicology, University of Guelph

Previous awards

1994 H.E. Sonnenberg.* University of Guelph, Guelph, ON, D.J.A. Kelly.* Queen's University, Kingston,ON
M.J. Faber. University of Guelph, Guelph, ON
1993 C. Flessas. University of Montreal, Montreal, QC, M.E. McMaster. University of Guelph, Guelph, ON
1992 D.M.A. Monita. University of Calgary, Calgary, AB, K. Kidd. University of Alberta, Edmonton, AB
1991 M.H. Murdoch. University of Guelph, Guelph, ON, C. Vanier. University of Quebec, Montreal, QC
1990 R. Lanno. University of Waterloo, Waterloo, ON

*Co-winners

WORKSHOP PROCEEDINGS/COMPTE RENDUS D'ATELIER

The Proceedings of each Annual Aquatic Toxicity Workshop have been published in a series of Technical Reports listed below. These Proceedings are generally provided to each Workshop participant, and are also sent to various libraries, government departments and other agencies that are listed on the Department of Fisheries and Oceans technical reports mailing list. Copies of most Proceedings, as photocopies or fiche, are available for a charge from Micromedia Limited, 240 Catherine Street, Suite 305, Ottawa, ON, K2P 2G8 (613-237-4250). Their catalog numbers (MLCN) are listed below where applicable.

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Proceedings of the Twentieth Annual Aquatic Toxicity Workshop: October 17-21, 1993, Quebec City, Quebec. Edited by R. Van Coillie, Y. Roy, Y. Bois, P.G.C. Campbell, P. Lundahl, L. Martel, M. Michaud, P. Riebel and C. Thellen. Can. Tech. Rep. Fish. Aquat. Sci. 1989: 331 p. (MLCN: 95-00001).

Proceedings of the Nineteenth Annual Aquatic Toxicity Workshop: October 4-7, 1992, Edmonton, Alberta. Edited by E.G. Baddaloo, S. Ramamoorthy and J.W. Moore. Can. Tech. Rep. Fish. Aquat. Sci. 1942: 489 p. (MLCN: 94-02230).

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Proceedings of the Ninth Annual Aquatic Toxicity Workshop: November 1-5, 1982, Edmonton, Alberta. Edited by W.C. Mckay. Can. Tech. Rep. Fish. Aquat. Sci. 1163: 243 p. (MLCN: 84-3262).

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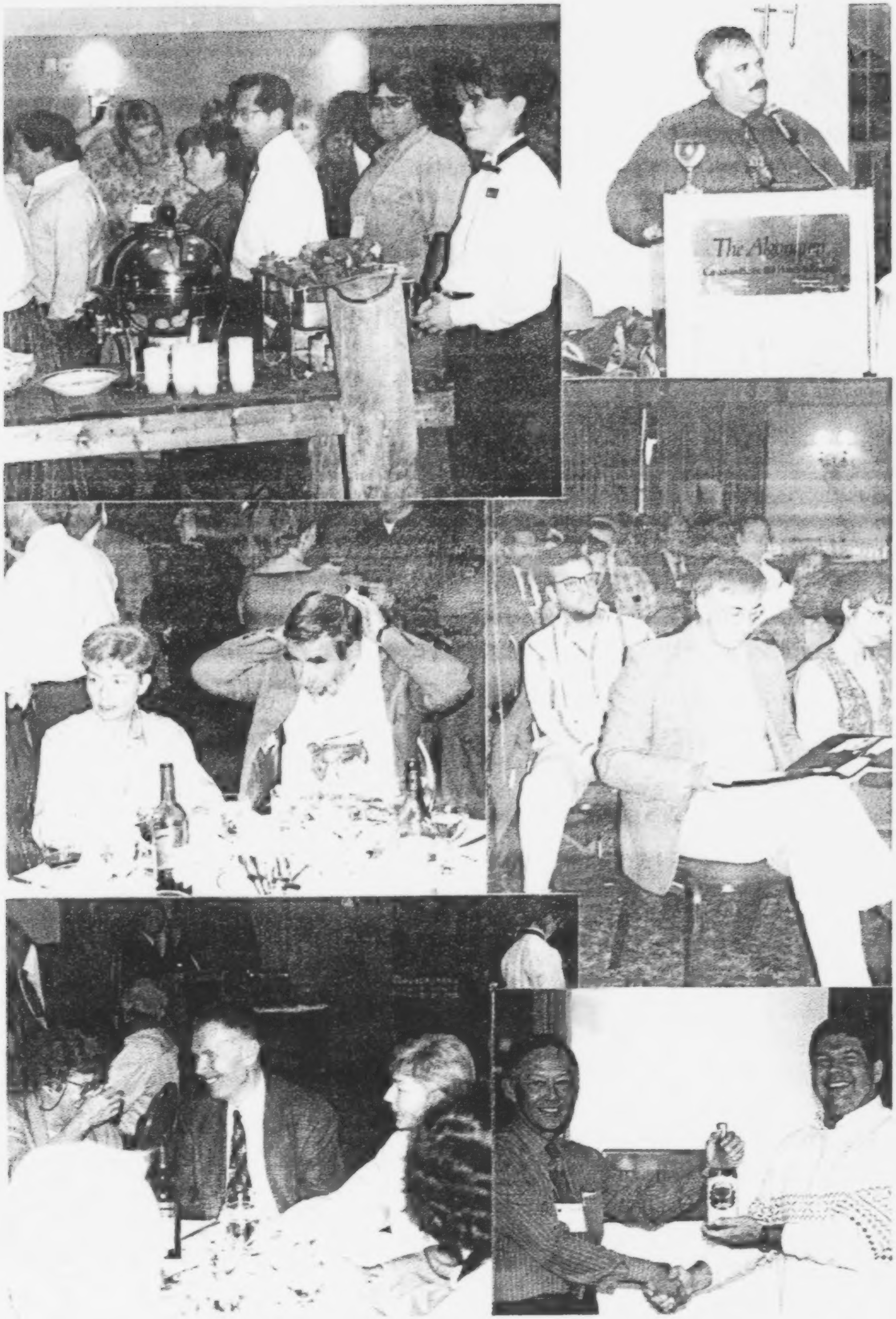
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